Effects of saline water irrigation on growth, persistence and nutritional quality of *Medicago arborea* and *Atriplex* species grown on different soil types

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By

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Table of contents

Abbreviations

ABA	Abscisic acid
ADF	Acid detergent fibre
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATPase	Enzymes catalyse the decomposition of ATP into ADP and Pi
CaM	Calmodulin
CBLs	Calciuneurin B-like protein
CCC	Cation chloride cotransporter
CDPks	Calcium–dependent protein Kinases
CIPKs	CBL interacting protein Kinases
CMLs	Calmodulin-like proteins
CP	Crude protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DM	Dry matter
DMD	Dry matter digestibility
DOMD	Dry organic matter digestibility
EC	Electrical conductivity
ECse	Electrical conductivity of soil saturate paste
ESP	Exchangeable sodium percentage
Fv/Fm	Maximal quantum efficiency of PSII
GORK	Guard cell K^+ outward-rectifier
g_s	Stomatal conductance
GUS	β-glucuronidase
LIX	Liquid ion exchanger
ME	Metabolisable energy
MIFE	Microelectrode ion flux measurement
NDF	Neutral detergent fibre
NSCC	Non selective cation channel
PCD	Programmed cell death
ROS	Reactive oxygen species
SOS	Salt overlay sensitive

Abstract

Agricultural crop production relies heavily on irrigation. Due to the lack of sufficient fresh water, the usage of low quality (saline) water is gaining momentum in many arid and semi-arid regions of the world. However, there are some concerns that using saline irrigation water may be an environmentally non-sustainable practice with potential to cause irreversible soil degradation. In addition to this, there is a lack of information on physiological changes in the plant when irrigated with saline water over a long-period of time and the impact of long term saline water irrigation on soil chemical properties and nutritional qualities of plants grown under such conditions. The major aim of this work was to investigate the effect of different levels of salinity in irrigation water and a range of irrigation regimes on the performance of *Atriplex halimus, Atriplex lentiformis* and *Medicago arborea* grown in different soil types. This study also investigated the effect of saline water on selected soil chemical properties and nutritional qualities of these species. By doing this the following objectives were addressed:

1. To understand the effect of saline irrigation on changes in the physiological characteristics and ion accumulation in plants in the context of different growing media (soil types - clay, duplex (texture-contrast) and sandy loam soil) and the amount of irrigation water applied.
2. To monitor changes in plant physiological characteristics and ion accumulation patterns over time of exposure to salinity during the growing period.
3. To determine the effects of quality and quantity of saline water on plant biomass production on different soils.
4. To investigate the effect of saline water irrigation on soil salinity and some selected chemical properties in the soil profile.
5. To understand the impact of saline water on some selected nutritional qualities of plants and to explain nutritional quality difference between plant species.

A series of glasshouse experiments were conducted investigating plant gas exchange and photosynthetic characteristics and relating them to plant tissue ionic profiles. The stomatal conductance (g_s) of *M. arborea* was found to be reduced by 41% and 74% of control at 8 and 16 dS/m salinity, respectively, whereas no such effect was apparent in *Atriplex lentiformis*. In both plant species leaf ions (Na^+ and Cl^-) content was increased with increasing water salinity (ECw) but a significant effect was seen only in a sandy loam soil.

This indicates that both osmotic effect and ionic toxicity impacted on the physiological performance of *M. arborea* but *A. lentiformis* plants were insensitive to both components of the salt stress. Plant growth in a sandy loam soil was poor compared to a clay soil, indicating that soil texture and structure may have a significant role in the cation exchange process under saline conditions.

Monthly monitoring of plant physiological characteristics and relating them to soil physical and chemical characteristics gave some further insights into effects of soil types and saline irrigation regimes on the performance of both species. Both chlorophyll content and Fv/Fm chlorophyll fluorescence characteristics were stable and did not change with time for all soil types, salinity levels and irrigation regimes. A general decline in the stomatal conductance (g_s) was observed in both *A. lentiformis* and *M. arborea* as the stress progressed, with the decline being much less pronounced in *A. lentiformis* (i.e. 40 vs 55 % over the 4 months at 16 dS/m ECw salinity). Plants grown in clay and duplex soils showed a greater reduction in g_s compared with a sandy loam soil in both species. In addition, the leaf Na^+ content in *A. lentiformis* remained constant over the entire duration of the experiment (5 months) regardless of EC_w and irrigation levels, while in *M. arborea* a continuous accumulation of Na^+ in leaves was observed. Leaf K^+ content in *A. lentiformis* was steady for the clay soil but declined when grown in a sandy loam (3.2-fold reduction over the 5-month period at 16 dS/m EC_w salinity). Overall, it is concluded that higher reliance on organic osmolytes (hence, associated carbon costs) for osmotic adjustment, poor K^+ retention, a lack of efficient vacuolar Na^+ sequestration, and higher reduction in stomata aperture in *M. arborea* are the main factors that explain poor performance of this species under long-term (5 months) salinity treatments in high salinity conditions (ECw 16 dS/m). On the contrary, *A. lentiformis* had superior performance in each of the above components and showed no time-dependent changes in any of the measured characteristics.

The above conclusions obtained in glasshouse experiments were further supported by a field trial. *Atriplex halimus* and *A. lentiformis* were shown to benefit from the application of saline water (up to 16 dS/m) at 800 mm/year irrigation rate in addition to natural rainfall (on average 500 mm/year), without any detrimental impact on plant growth and biomass production. On the other hand *M. arborea* could only be irrigated with up to 8 dS/m in the same conditions. The strongest reduction in *M. arborea* yield was observed in clay dominated soil under high irrigation regimes and was most likely associated with a transient waterlogging.

Glasshouse data demonstrated that soil salinity (EC_{se}) increased with increasing salinity of irrigation water (EC_w). Regardless of irrigation regime, EC_{se} was higher in clay dominated soil (clay and texture-contrast soil) compared with sandy loam soil. For the 16 dS/m EC_w treatment, clay soil salinity did not exceed the salinity level of irrigation water when irrigated at a high irrigation rate, and it was about 1.5 fold higher compared with low irrigation treatment. In the sandy loam soil, EC_{se} values were only half the value of EC_w. On the other hand, field experiment data showed a sharp increase of EC_{se} in the soil profile (including the top sandy soil) after 2.5 years of application of saline water (16 dS/m) but the value was less than the ECw. In the high salinity condition, topsoil (0 - 10 cm) total organic carbon (TOC) was significantly reduced. Whilst, in the upper 50 cm depth, both the exchangeable Na^+ and its ratio to total base cations (ESP) were significantly increased but the individual exchangeable base cations ($Ca^{2+,}$ Mg^{2+}, K^+) were significantly decreased. They also show that the Na^+ in the saline waters causes differential leaching of base cations from the rooting zone to deeper in the soil.

In the last part of this project saline water irrigated *A. halimus, A, lentiformis and M. arborea* forage samples were collected after 3 years of field growth and analysed by wet chemistry methods for selected nutritional values. The measured nutritional parameters varied between species with *M. arborea* having a higher calcium (1.6%) but others minerals were higher in *Atriplex* species. In *Atriplex* species calcium content increased with increasing EC_w but was reduced in *M. arborea.* Regardless of irrigation levels and plant species, ash percentage increased from 16.8 to 17.8 % and Na^+ content from 3.7 to 4.3 % when salinity of irrigation water increased from 0.8 to 16 dS/m but K^+ content decreased from 1.7 to 1.5 % under the same condition. Collectively, nutrient detergent fibre (NDF) values of plants were reduced from 35 to 33 % DM when the EC_w increased from 0.8 to 16 dS/m.

In summary, this work has shown a possibility of sustainable production of *M. arborea* and *Atriplex* species using up to 8 and 16 dS/m saline water, respectively, with an irrigation rate of 800 mm/year on a Brown Sodosol in a 500 mm annual rainfall region. As the topsoil salinity increased with irrigation water salinity and some Ca^{2+}, Mg^{2+} and K^+ were leached down to the subsoil from the active root-zone area in the soil profile. Such a practice may be not recommended to be used on prime agricultural land. However, for already salt-contaminated land not suitable for conventional farming the usage of high irrigation saline water regimes is likely to be beneficial, as at no treatment conditions did soil EC_{se} exceeded that of EC of the irrigation water. This study has also provided further insights into halophyte physiology demonstrating (in long-term experiments) that the better ability of halophyte

species to cope with salinity is related to their much higher reliance on inorganic osmolytes for osmotic adjustment. This strategy allows these plants to avoid the yield penalties experienced by glycophytes due to a need to redirect a major pool of photosynthates for *de novo* synthesis of organic osmolytes. Finally, this study has also showed that the nutritional value of these plant species will not be affected by the saline water irrigation with the exception of increased shoot Na^+ ion content.

Chapter 1: General introduction

Soil salinity is increasingly contributing to land degradation worldwide with it currently affecting approximately 7% of the earth's land surface (Flowers et al., 1997). Salinity is one of the most significant land based environmental problems in the world affecting irrigated agriculture, including Australia where dryland salinity is also a very significant issue. In Australia salinisation affect about 30% of the land area (Rengasamy, 2006). It is estimated that about 20% of cultivated lands worldwide are affected by high levels of land based salinity (Munns, 2005). Of these, about 100 Mha of land have become saline due to irrigation (Ghassemi et al., 1995; Pessarakli and Szabolics, 1999). Globally, irrigated land represents only 16% of the total arable land but they account for 44% of all crop production. In developing countries, 21% of arable land is irrigated and they account for 60% of all cereal production (Alexandratos and Bruinsma, 2012).

The world population is projected to reach 8.1 billion in 2025 and 9.6 billion in 2050 (United Nations, 2014) implying that global food production will need to increase by up to 70% by this time. This could be achieved with the expansion of the irrigated land area (Alexandratos and Bruinsma, 2012) however, there are other options to increasing food production and sustainibility of agriculture as suggested by McKenzie and Willams (2015). Also, as food demand increases expansion of agricultural land area, both rainfed and irrigated, is projected to increase, particularly across central and southern Africa (UNEP, 2009). It is estimated that irrigated land could expand by 20 Mha (or 6.6 %) from 2005 – 2050 nearly all of it in the developing countries (Alexandratos and Bruinsma, 2012). But a key question is; whether fresh water resources alone can meet the irrigation water demand in future? Agriculture already accounts for about 70% of the freshwater withdrawals worldwide (FAO, 2011) and irrigation and its expansion is one of the main factors causing global freshwater scarcity (Turral et al., 2011). The shortage of quality fresh water is already limiting irrigated agricultural production especially eastern Mediterranean countries and Australia (Ventura et al., 2014; Schacht and Marschner, 2015). So, what are the possible solutions to this pressing problem? One answer is in the use of marginal quality water for saline agriculture on areas of existing saline and/or sodic soils.

Saline agriculture is an alternative option for farming where land and water is not suitable for the conventional agriculture. When this system is undertaken properly saline soil and water become useable resources rather than a burden or waste issue. The benefits of

developing *saline agriculture* enable food, fodder, and biofuel production from degraded lands and low quality water resources (Yamaguchi and Blumwald, 2005; Panta et al., 2014). Saline agriculture might become a viable strategy of future farming to help deal with the growing food and biofuel demands of an increasing global population (Shekhawat et al., 2006). In the near future fresh water availability for conventional farming will further also shrink due to direct impact of global climate change on freshwater resources (Nelson et al., 2009). This will force farmers into using lower quality waters for food and fodder production in irrigated areas. On the other hand, gas and oil industries and municipality waste treatment plants are generating large volumes of various lower quality waters (Shannon 1997; Miyamoto et al., 2005, Gerhart et al., 2006; Glenn et al., 2009) with sodium being the dominant cation and commonly being discharged into waterways (Gerhart et al., 2006). However if these could be used in sustainable ways they could substitute for a proportions of the freshwater used in current irrigation in suitable soils and climatic zones.

As irrigated land yields twice as much as rainfed land (UNEP, 2009), it is highly unlikely that the irrigated agriculture will be abandoned readily, despite the emerging challenges. Thus, salinity issues will both remain and expand as a key threat to the global food production in the 21st century when more food production is needed from limited agricultural land (Shabala and Munns, 2012).

Globally 11 percent of the irrigated areas are already affected by some degree of salinisation (FAO, 2012) and their continuous expansions in developing countries are posing serious threats to farming of conventional croplands (Panta et al., 2014). The severity of land degradation due to salinity is expanding at the rate of 2,000 – 4,000 ha/day in irrigated agricultural systems of the arid and semi-arid regions (Shabala, 2013; Qadir et al., 2014). This means in future there will be a "double blow" in farming enterprises due to the shortage of freshwater and loss of land and production from the effects of salinity. In addition to this it has been estimated that salt induced land degradation is already costing as much as US$27.3 billion in 2013 (Qadir et al., 2014). These challenges need urgent research and development for suitable solutions. These solutions also need to be economically viable to meet the future's food demand while making farmers profitable and sustainable. One of these options is the usage of salt-tolerant glycophytic and halophytic plants for food, forage and fodder production, either by utilising saline land or by saline water irrigation to ease the pressure already mounted on the conventional agricultural system. However, there is a challenge to use these saline waters for irrigation purposes because of the possibility of secondary salinity problems in soil profiles and shallow groundwaters (Glenn et al., 2009). Also human and

animal health and the environment health need to be protected from any undesirable or excessive constituents found in untreated wastewater (Qadir et al., 2010).

Salinisation is the process of increasing concentration of total dissolved salts (TDS) in soil and water either due to natural process (primary) or anthropogenic actions (secondary) (Ghassemi et al., 1995). The process of secondary salinisation accelerates if saline water is used for irrigation (Barrett- Lennard, 2002; Lambert and Turner, 2000), especially in clay dominated soil. The effects of salinisation can be severe; the short term effects being loss of agricultural productivity and the longer term effects being permanent damage of soil properties if precautions are not taken to ensure sustainable principles are applied. For example, soil salinity and sodicity were found to increase due to the use of saline water (Ganjegunte et al., 2005; Johnston et al., 2008; Clark and Veil, 2009). However, it is believed that use of high volumes of water onto the porous soils, i.e., those containing a high proportion of sand, prevents salt build-up in the root zone and this irrigation technique can be a key strategy for saline agriculture (Glenn *et al.,* 1999, 2009). Not only soil texture and structure but also other factors such as soil hydrological properties, depth to the watertable, micro-climatic conditions i.e., the rate of evapotranspiration, all may have significant effect on this process. Also, previous studies have shown that use of saline water in poorly drained soils such as unstructured clays can intensify the deterioration of soil physical properties due to dispersion of clay particles and hence further reduced infiltration and drainage, increased potential of soil erosion, crusting and hard setting (Sharma and Manchanda, 1996; Sumner et al., 1998; Bauder and Brock, 2001; So and Aylmore, 1993; Halliwell et al., 2001; Warrence et al., 2002; Tarchitzky *et al*., 2007; Clark and Veil, 2009). However, to a lesser extent its effect on soil chemical properties is explained (Tedeshi and Dell' Aquila, 2005; Jalali *et al*., 2008; Biggs and Jiang, 2009), so it needs to be researched and monitored. Similarly, in the past many halophytic research activities were focussed on yield, comparison of nutritional value of these plants to conventional forages (Watson and O'Leary, 1993; Masters et al., 2007; Khan et al., 2009) and animals performance and meat quality under halophytic feed (Gihad and El Shaer, 1994; Miyatmoto et al., 1994; Norman et al., 2004; Abu-Zanat and Tabbaa, 2006; Khan and Ansari, 2008 ; Khan et al., 2009; Al- Shorepy et al., 2010). There have been few studies on the effect of growth conditions on nutritional quality of the forge (Masters et al., 2009). Thus, the knowledge of changes in the soil chemistry under saline water irrigation and effects of growth conditions on plant nutritional quality may be a key for the success of saline agriculture.

As salinity is difficult to manage because of their long term effects on soil and water resources, its impact at the farm level is the loss of the production and income. This indicates the need for less expensive and approachable salinity management strategies for land managers. Out of the several approaches proposed for saline land management, the least expensive approach seems to be the cultivation of salt tolerant crops, pasture and fodder species. Thus if salinity problems exist or are expanding then the need for alternative profitable saline tolerant crops and farming systems which are adapted to such environments is required. That's why the concept of saline agriculture was begun as is a system where salt-tolerant and salt loving plants are utilised effectively. Research in this field is not only helpful for revegetation of the drought and salt affected land but also show the potential of use of marginal or salty water for economic uses. In this study, two halophytic species, *Atriplex lentiformis* and *Atriplex halimus*, and one salt-tolerant glycophytic, *Medicago arborea*, all being forage shrubs were selected because of their utility as an alternative source of forage for livestock in arid and semi-arid environment.

Halophyte plants possess a remarkable ability to adapt to and thrive in saline conditions (Glenns et al., 1999). However, although they can grow well under certain growth conditions their performance cannot be extrapolated to all saline. In this study the selected plants *Atriplex lentiformis*, *Atriplex halimus* and *Medicago arborea* were subjected to different soil textural set-ups in the glasshouse and field experiment and irrigated with differetnt quantity and quality of irrigation waters.

This research project was part of a larger ARC Linkage Grant and was initially designed to address the issue of large amounts of saline water produced from Coal Seam Gas (CSG) operations in the Surat basin area of Queensland. The irrigation rates selected and the salinity levels were set to match those of the CSG industry which required the disposal of these waters on a regular basis. In 2013, CSG produced approximately 18,500 megalitre of wastewater with salinity level of up to 17 dS/m (IESC, 2014). This huge amount of water either can be reinjected (treated or untreated) into aquifers and/or added to the existing water resources to support agriculture (IESC, 2014). Although treatment of these waters can reduce the hazard levels so as to allow their safe discharge to local water ways, this is an expensive operation. Also the successful operation of these treatment facilities can depend upon several factors such as the volume of water involved, and capacity of the treatment plants. In addition to this, the cost of construction and operating such plants is substantial, and so is there "carbon footprint" if using conventional power sources. For example the cost of construction of one "standard size" desalination plant to dispose of 5 ML/day of saline water was

estimated to be about A$17.5M, with a additional A$6M per annum operation cost (Panta et al., 2015). Thus, economically viable alternatives for CSG waters to allow agricultural use these waters for forage production in marginal lands warrant investigation. If these waters could be used for irrigation their impacts will vary depending on local soil types, quality and quantity of the saline CSG water used. Although past research on *Atriplex* species have shown some knowledge on impact of saline water on plant and soil (Jordan et al., 2009; Soliz et al., 2011), there are still many unanswered questions. For example, what are the effects of quality and quantity of saline water on *Medicago arborea* and *Atriplex species* grown in different soil types? What are the major impacts of long term saline water irrigation in the soil chemical properties? What roles does different concentration of saline water play in tissue nutrient content of the plants grown in those environments? Those sorts of questions are the basis of this investigation and through these evaluations the suitability of plants, soil types, water salinity levels, and economic use of plant for saline agriculture were determined.

This thesis consists of eight chapters. Chapter 2 provides an extensive review of the literature describing saline (halophyte) agriculture and how these salt tolerant plants can be utilised for various purposes. Chapters 3 and 4 describe plant performance and kinetics of salinity effects on *Atriplex lentiformis* and *Medicago arborea* plants grown in different soil types and irrigation regimes in glasshouse conditions. Chapters 5, 6 and 7 summarise results of a major field experiment of *Atriplex lentiformis*, *Atriplex halimus* and *Medicago arborea* plants irrigated with different levels of saline water and irrigation rates in texture-contrast soil i.e., Brown Sodosol (Isbell, 2002). These chapters examine the effects of irrigation water salinity on plant performance, soil chemical properties and nutritional quality of plants under investigation. In Chapter 8, the findings of previous chapters are brought together and discussed in the light of above aims and unanswered questions in this study.

Aims of the research

The aims of this research were four fold:

1. To elucidate the effects of the quality and quantity of saline water on plant biomass production in different soils under both glasshouse and field conditions.
2. To provide insights into physiological mechanisms conferring plant adaptation to saline environment for plants grown in different soil types and under different irrigation regimes.
3. To investigate the effect of saline water irrigation on soil health by monitoring long-term changes in some selected chemical properties in the soil profile to 3 m.
4. To understand the impact of saline water on nutritional qualities of plants.

The above aims were addressed in a series of glasshouse and field experiments conducted at University of Tasmania and the University Farm near Cambridge, Tasmania, between 2011 and 2015.

Chapter 2: Literature review

Halophyte agriculture: success stories*

Suresh Panta[1], Tim Flowers[2], Peter Lane[1], Richard Doyle[1], Gabriel Haros[3], and Sergey Shabala[1]

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Abstract

The world's food production will need to increase by up to 70% by 2050 to match the predicted population growth. Achieving this goal will be challenging due to the decreased availability of arable land, resulting from urbanization and land degradation. Soil salinity is a major factor contributing to the latter process. While some improvement in crop yields in saline soils may be achieved as a consequence of single gene transfers, the real progress may be achieved only via a painfully slow "pyramiding" of essential physiological traits. Given the time constraints, a safer solution to meet the 2050 challenge may be to find alternative crop and forage species for farming in salt-affected conditions and to restore salt-affected areas. This review focuses on the suitability of halophytes to become important components of 21st century farming systems. We provide a comprehensive summary of the current use of halophytes for human food consumption, for forage and animal feeds, as oilseed and energy crops, and for desalination and phytoremediation purposes. We argue that the use of halophytes may be a viable commercial alternative to ease pressure on the requirement of good quality land and water for conventional cropping systems and the utilization of land degraded by salinity.

Key words: salinity; desalination; phytoremediation; forage species; energy crops; saline irrigation; sodium removal; saline agriculture

2.1. Meeting the 2050 challenge in a 'salinised world'

The world's human population is expected to reach 9.1 billion by 2050, and global food production will need to increase by up to 70% by this time to match this growth (FAO, 2011). This will require an average annual increase in grain production of 44 million tonnes per year for the next 40 years (Tester and Langridge, 2010), equating to an additional 38% increase over historical trends in production. Achieving this goal is urgent and will become increasingly challenging due to the decreasing availability of arable land as a result of urbanisation and soil degradation. The acuteness of this problem is further exacerbated by the challenges imposed by changes to the global climate that are expected to increase the frequency and severity of drought stress (and, hence, a need for high quality irrigation water) in many regions world-wide (Setter and Waters, 2003), severely affecting crop production. Furthermore, as part of global response to climate change there will be increasing competition between crop and biofuel production. Consequently, substantial changes in both agronomic and breeding practices are required. On a global scale, the overall loss in value of food and fibre production due to abiotic stresses is estimated at US$120 billion p.a. (http://www.fao.org/docrep/008/y5800e/ Y5800E06.htm).

Soil salinity is an increasingly contributing to land degradation worldwide, with approximately 7% of the earth's land surface having salt-affected soils while sodium-affected soils are even more widespread (Flowers et al., 1997). Salinisation is the process of increasing the concentration of total dissolved salts in soil and water either due to natural processes (primary salinisation) or anthropogenic actions (secondary salinisation) (Ghassemi et al., 1995). The process of secondary salinisation is exacerbated by the use of saline ground water and poor quality waste water for irrigation, as well as by clearing deep-rooted forest lands for pasture and crop production (Lambert and Turner, 2000; Barrett-Lennard, 2002). Globally, irrigated land represents only 15% of the total cultivated land but produces one third of the world's food (Munns, 2005). On average, it is estimated that about 100 Mha of land have become saline due to irrigation (Ghassemi et al., 1995; Pessarakli and Szabolics, 1999), so about 11% of the world's irrigated areas are already affected by some degree of salinisation (FAO, 2012). Alarmingly, the amount of salt-affected land and its continuous expansion is highest in some of the most populated and economically challenged countries such as Bangladesh (1 Mha; Hossain, 2010), India (7 Mha; Vashev et al., 2010) and Pakistan (3 to 6 Mha; Qureshi et al., 2008; Vashev et al., 2010), posing a serious threat to sustainable agricultural production. One of the worst cases of salinisation is in the Aral Sea Basin in

central Asia (Kazakhstan, Kyrgyz Republic, Tajikistan, Turkmenistan, and Uzbekistan) (Kijne 2005; Qadir et al., 2009), where up to 50% of the irrigated area is affected by salinity and/or waterlogging; thousands of square kilometres of irrigated land were degraded when virgin lands were converted into irrigated agricultural land (Qadir et al., 2009). It is not, however, only the poorer countries that are affected by salinisation: the economically well-developed countries are also susceptible to secondary-induced soil salinization (Yensen, 2006). For example, a survey conducted in Australia in 2002 showed that 2 Mha of agricultural land were showing signs of salinity, and nearly 820,000 ha of land were considered as unsuitable for commercial crop production (Australian Bureau of Statistics, 2004). On a global scale, it has been estimated that every minute 3 ha of currently arable land becomes unproductive due to secondary-induced salinisation (Zhu et al., 2005), and it is estimated that between 10 and 20 Mha of irrigated land deteriorates to zero productivity each year (Hamdy, 1996; Choukr-Allah, 1996).

The majority of the crop and forage species used in modern agriculture are salt sensitive (glycophytes) and can handle only a very limited concentration of salt in their growth media. Once salinity in the soil solution exceeds a certain level, productivity is reduced to the extent of becoming commercially not viable (Table 2). For most species, a 10% yield decrease is observed as the salinity, measured by the electrical conductivity of a soil solution increases over the 4 to 8 dS/m range[1] although some species are much more sensitive. For example, the grain yield of maize (*Zea mays* L.) may be reduced by 21% for each unit increment of electrical conductivity in the irrigation water above a value of 1.7 dS/m (Blanco et al., 2008). On the other hand, the growth of many halophytic species is stimulated within a salinity range of 15-25 dS/m (Rozema and Schat, 2013).

As salinity tolerance is a highly complex trait, both physiologically and genetically, it is hardly surprising that progress in crop breeding for salinity tolerance has been very modest during most of the 20th century, with no truly salt tolerant cultivars delivered to farmers' fields (Flowers, 2004). While transgenic approaches have been advocated since the early 1990's (Tarczynski et al., 1993), and there were to the early March 2013 more than 430

[1] It is important to note that while salinity is often characterised by the conductivity of a saturated paste (ECe), plants do not grow at the soil:water ratio of saturated pastes. Rather, they respond to the salinity of the soil solution (the ratio of soil salinity to soil water existing in the soil at any given time). The EC of the soil solution is approximately twice the ECe when the soil is at field capacity (drained upper limit) and about four times the ECe when the soil is at permanent wilting point (Richards 1954). The severity of salinity can also be expressed in terms of the salinity of the irrigation water (ECw). The EC of the soil solution will equal the ECw immediately after irrigation, but will become more concentrated than this as the soil dries with evaporation and plant transpiration. For practical purposes, as a rule of thumb electrical conductivities can be converted to salt concentrations knowing that for NaCl solutions 1 dS/m $\approx$ 10 mM NaCl (Richards 1954).

papers involving transgenic plants produced with the aim of enhancing salt tolerance, very few of these products have been tested in field. We have evaluated 17 papers in which field trials (search for 'field' in the Web of Science) were mentioned in the title or abstract of papers in which 'salt or salinity and transg*' also appeared in the paper. Of these just seven reported the responses of transgenic lines to salinity in field trials. In 2004, two lines of wheat (*Triticum aestivum*) transformed with a Na^+/H^+ antiporter (*AtNHX1*) from *Arabidopsis thaliana* (see Table 1**)** were grown alongside a cultivar (Hesheng 3) in plots in normal or saline soil (Xue et al., 2004). The transformed wheat produced similar yields in the non-saline soil, but improved (33-50%) yields compared with the cultivar in saline soils (the salinity was reported as a mean electrical conductivity of soil saturated pastes (EC_e) from before and after harvest: values were 10.6 and 13.7 dS/m. Wheat transformed to contain a

Table 1. Summary of results of field trials of transgenic plants subjected to saline conditions.

Species	Transformed with	Comments	Reference
Triticum aestivum	*AtNHX1*	Improved yield [a]	Xue et al. (2004)
Triticum aestivum	*AhCMO*	Improved yield; no detail of how salinity was measured	He et al. (2010)
Triticum turgidum ssp. durum	*TmHKT1;5-A*	Improved yield in one of three saline sites	Munns et al. (2012)
Populus tomentosa	*mtl*D	No beneficial effect on yield of field-grown plant reported	Hu et al. (2005)
Populus alba x P. berolinensis	*JERFs*	Improved growth (biomass); salinity level uncertain	Li et al. (2009)
Gossypium hirsutum	*CMO*	Salinity level uncertain; no non-saline control	Zhang et al. (2009)
Trifolium alexandrinum	*HARDY*	Improved yield under saline conditions	Abogadallah et al. (2011)
Morus indica	Hva1	Phenotype only; no actual yield improvement reported	Checker et al. (2012)

[a] By "improved yield" we refer to yield of transgenic plants grown under saline conditions compared with control.

choline dehydrogenase has also been field tested and yields were shown to be improved in a field (He et al., 2010), although there reported soil salinity levels are confusing and not clearly defined. There have also been reports of transformed poplars – *Populus tomentosa* transformed with a mannitol-1-phosphate dehydrogenase (Hu et al., 2005) and of a hybrid poplar (*Populus alba x P. berolinensis*) transformed with a jasmonic ethylene responsive factor gene from tomato (Li et al., 2009) - being tested in the field. For the trial of *P. tomentosa*, there was no detail of any salt stress or growth or yield data and for the hybrid poplar, growth was improved, but there was little detail on the salinity were provided. The yield of cotton (*Gossypium hirsutum*) transformed with choline monooxygenase was reportedly "significantly higher than that of non-transgenic plants under salt-stressed field conditions" (Zhang et al., 2009), although there was no detail of the salinity in the plots or a non-saline control. *Trifolium alexandrinum* has been transformed with an AP2/ERF gene (*HARDY*) from Arabidopsis and shown to have improved fresh and dry weights in a trial that involved drought in a saline field (Abogadallah et al., 2011); again, without knowing what kind of extract was used on the soil, the salinity measures in this study are uninterpretable. Although mulberry (*Morus indica*) transformed with a late embryogenesis abundant gene from barley has been grown in fields, no growth data were published related to salinity (Checker et al., 2012). Finally, Munns et al. (2012) have shown that the presence of the TmHKT1;5-A gene, encoding a Na^+-selective transporter located on the plasma membrane of root cells surrounding xylem vessels, significantly reduced leaf Na^+ concentration and increased durum wheat grain yield by 25% compared to near-isogenic lines without the gene Nax2 locus. Altogether, three saline blocks were tested in a field, with average EC_e (electrical conductivity of a saturated soil extract) values of 12.9, 11.4 and 14.8 dS/m. Of these, a moderate yield improvement (1.6 t/ha compared with 1.3 t/ha for non-transformed plants) was reported only for the one block, while in other blocks no significant difference was found between transformed and non-transformed plants. Moreover, even in the 'successful' block the grain yield of transformed plants under saline conditions (EC_e = 14.8 dS/m) was only 50% of the control (EC_e = 2.3 dS/m). So, even where claims for improved performance are substantiated, the results should be viewed with a degree of caution.

In light of the above, it becomes more and more clear that while some improvement in yields in saline soils may be achieved as a consequence of a single gene transfer (the 'silver bullet approach' advocated by molecular breeders), real progress may be achieved only via a painfully slow pyramiding of essential physiological traits (Flowers and Yeo, 1995). Given the time constraints (see the first paragraph), an approach to breeding for salt tolerance that

involves putting 'all eggs in one basket' may be unwise. Other options for combating salinisation, such as finding alternative crops for farming in those conditions and the restoration of salt-affected areas should be considered. This review focuses on the suitability of halophytic species to become important components of 21st century farming systems. We argue that the use of halophytes may be a viable commercial alternative to ease pressure on the requirement of good quality land and water for conventional cropping systems and the utilization of land degraded by salinity.

2.2. Halophytes as a possible alternative to glycophytic crops

Halophytes are naturally evolved salt-tolerant plants that represent at most 2% of terrestrial plant species (Flowers and Colmer, 2008). They have the ability to complete their life cycle in a NaCl-rich environment where almost 99% of salt sensitive species die because of NaCl toxicity, and thus may be regarded as a source of potential new crops (Glenn et al., 1999). Although halophytes have been recognized for hundreds of years, their definition remains equivocal (Flowers and Colmer, 2008), ranging from a Wikipedia-style description of "*naturally salt-loving plants*" to plants whose "*growth benefits from having some substantial amounts of salt in the growth media*" (Shabala and Mackay, 2011) to plants that "*have an ability to complete the life cycle in a salt concentration of at least 200 mM NaCl under conditions similar to those that might be encountered in the natural environment*" (Flowers et al., 1986). Other classifications of halophytes have been suggested that are based on the characteristics of naturally saline habitats, or the chemical composition of the shoots ('physiotypes'), or the ability to secrete ions ("recreto-halophytes") (reviewed in Flowers and Colmer, 2008).

Halophytic species possess a range of highly efficient and complementary morphological, physiological and anatomical characteristics to combat and even benefit from a saline environment (Flowers et al., 1977; Flowers and Colmer, 2008; Shabala and Mackay, 2011). The major hallmark of all halophytes is their ability to use inorganic ions such as Na^+ and Cl^- (available in the external media 'for free' – i.e. ions capable of being taken passively along the electrochemical gradient without spending any energy (ATP) to drive this process) for osmotic adjustment in their tissues when grown under saline conditions. Halophytes tolerate these ions because of their superior ability to sequester cytotoxic Na^+ in root and leaf cell vacuoles – a trait conferred by the constitutive expression of tonoplast Na^+/H^+ NHX exchangers (Apse and Blumwald, 2007) complemented by the efficient control of slow (SV) and fast (FV) vacuolar ion channels (Bonales-Alatorre et al., 2013ab) to prevent Na^+ from

leaking back into the cytosol. In contrast, glycophytic crops possess only a limited ability to use Na^+ for osmotic adjustment and rely heavily on *de novo* synthesis of organic osmolytes. Due to the high carbon cost of the process (Raven et al., 1985; Shabala and Shabala, 2011), the yield penalties are severe. As a result, the optimal growth of halophytes is observed between 100 and 200 mM (for dicotyledonous species) and between 50 and 100 mM (for monocots) NaCl in the soil solution (Flowers and Colmer, 2008) – the salinity range that makes key crops either non-viable (e.g. rice) or economically unproductive (Table 2). Thus, growth of halophytic plants would be a solution to manage brackish waters, whether naturally occurring or produced by various industries, which otherwise pose an environmental threat (Glenn et al., 1998; Watson et al., 1994); this would allow agriculture to move into desert areas, as well as into areas surrounding salt marshes and seacoasts.

While halophytes have long had a place in the diet of peoples across the world, scientific exploration of the potential for the use of halophytes as crops developed in the latter half of the twentieth century (reviewed in Rozema et al., 2013). Research on saline agriculture in Israel during the 1960s led to the development of a database of halophytes and their economic uses (Aronson, 1989). Up to now many halophytes have been evaluated for their potential use as crop plants (Miyamoto et al., 1996; Barrett-Lennard 2002; Barrett-Lennard et al., 2003; Reddy et al., 2008; Ruan et al., 2008; Qadir et al., 2008; Flowers et al., 2010; Rozema et al., 2013.), for the revegetation and remediation of salt and industrially polluted areas (Cambrolle et al., 2008; Lewis and Devereux, 2009), as floricultural crops (Cassaniti et al., 2013) and as biofilters for aquaculture effluents (Buhmann and Papenbrock et al., 2013). However, scepticism about their feasibility for use as alternative crop or forage species still remains. Although market development for saline agricultural products has only just started (Rozema and Schat, 2013), increased demand in the future is likely as the price of traditional crop products (e.g. vegetables) is increased due to the lack of good quality land and water for their cultivation (Rozema et al., 2013). This review summarises recent progress in this field and analyses the suitability and prospects of using halophyte species as food and forage species, oilseeds and biofuels, and for medicinal purposes, and phytoremediation.

Table 2. ECe values (dS/m) associated with 10% and 50% yield losses in selected crops.

Crop	Harvested area (million ha)[a]	Production (million tonnes)[a]	Yield (t/ha)[a]	EC_e (dS/m) associated with[b]	
				10% yield decrease	50% yield decrease
Rice (paddy)	164	723	4.40	3.5	6.8
Sorghum	35	54	1.53	7.7	9.6
Barley	49	134	2.76	9.8	17.5
Maize	170	883	5.18	2.5	5.5
Wheat	220	704	3.19	7.2	12.6
Sugarcane	25	1794	70.5	3.9	9.8
Potato	20	374	19.4	2.5	5.5
Sugar beet	5	271	53.7	8.5	15.0
Soybeans	103	261	2.5	5.5	7.2
Sweet potatoes	8	0.34	13.1	2.5	5.7
Cotton	35	77	2.19	9.4	16.8
Mung beans				2.1	3.9

Source: Adapted and modified from FAO STAT (2013)[a] and calculated by the method described by Steppuhn et al. (2005a,b)[b].

2.3. Halophytes as human food

At the present, 30 plant species provide 90% of human food with rice, maize, wheat and potato making up 50% of this value (Khan et al., 2006). At the same time, non-conventional crops (including halophytic crops) are seen as an alternative for farming in regions where only saline waters and saline soils are available and fresh water is considered a scarce resource (e.g. Middle East). The use of diverse halophytes as valuable cash crops could be a suitable option in such hostile environments. Moreover, even in countries not affected by salinity, consumption of products from some halophytic plants (e.g. quinoa) is growing rapidly (Fig. 1). In case of quinoa high market price caused an increasing production and export rather than increased consumption by the farmers of the Southern altiplano region of Bolivia (Jacobsen, 2011; 2012).

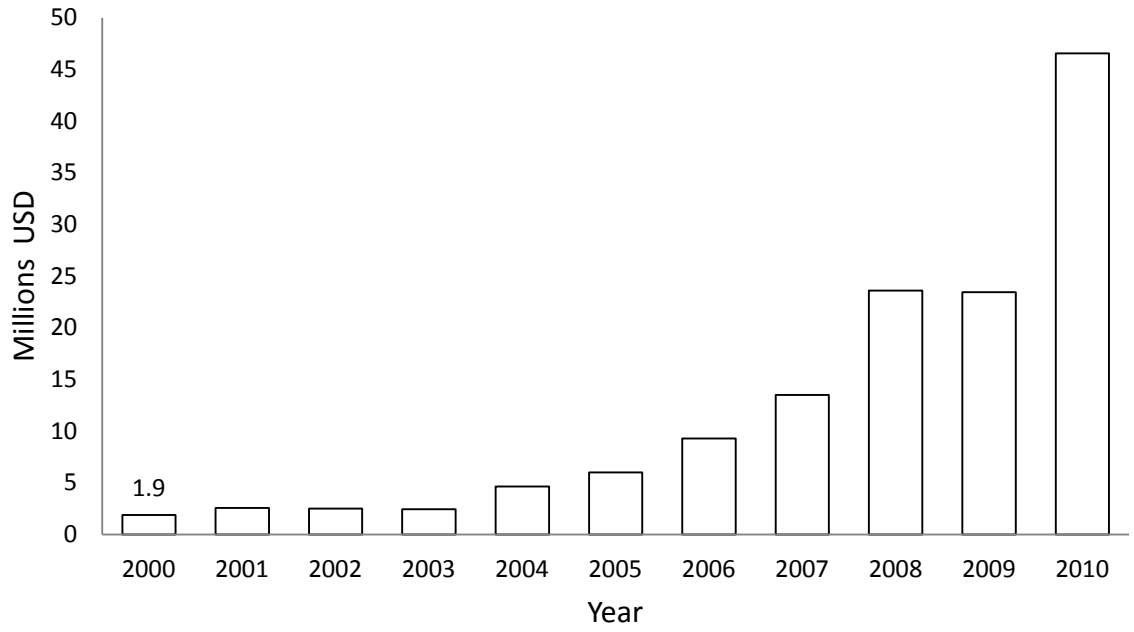

Fig. 1. Expansion of global export value of quinoa from 2000 to 2010 (FAOSTAT, 2013).

Halophytic plants have been used for human consumption for a long time (Table 3). For example, a perennial salt grass *Distichilis palmeri* was used as a food crop by the native South Americans for many centuries and as the main staple crop for the indigenous Cocopah people who lived along the lower Río Colorado in Mexico. This species has higher fibre content than wheat (8.4% vs 2.6%, respectively; Yensen et al., 1985) and is used for making biscuits and bread in various parts of the world (Yensen, 2006). This plant performs well even in flooded and hyper-saline condition (i.e. seawater salinities) and produces 1.25 t/ha of grain of high nutritional value (Pearlstein et al., 2012). Similarly, quinoa (*Chenopodium quinoa*) which is one of the staple foods of native South Americans can tolerate ECe values up to 40 dS/m (Adolf et al., 2013) and is regarded as an exotic food that is being sold at a premium price in European markets (Table 4). It has higher nutritive value than traditional cereals (Vega-Gálvez et al., 2010). Its seeds are rich in lysine (an essential amino acid), iron, magnesium, vitamin E, copper and phosphorus as well as being gluten free (James, 2009). Research conducted in temperate environments in Argentina showed that this crop has a grain yield potential of up to 5.2 t/ha (reviewed by Gómez et al., 2011). Similarly, pearl millet (*Pennisetum typhoides*) can tolerate irrigation water with EC_w > 30 dS/m and can be grown as a food crop with a seed yield of up to 1.6 t/ha (Jaradat, 2003). This yield is similar to that of wheat under dryland non-saline conditions.

The leaves of halophytes often have a taste similar to conventional salad crops. *Atriplex triangularis* leaves are similar to those of spinach (Islam et al., 1987) and are used for human consumption in the Netherlands, Belgium and Portugal (Leith et al., 2000). When grown under seawater irrigation (30 g NaCl/L) in Delaware (USA), *A. triangularis* yield was 21.2 t/ha on a fresh-weight basis (Gallagher, 1985). L*eaves of Salicornia bigelovii can be used as an alternative source of* omega-3 polyunsaturated fatty acids for human consumption. This species is being grown for the USA and European fresh produce markets (Zerai et al., 2010; Ventura et al., 2011). *S. bigelovii* has also been found to be a good source of the antioxidant β-carotene, with quantities ranging from 4.7 (Ventura et al., 2011) to 15.9 mg/100 g fresh weight (Lu et al., 2010). These values are similar or above those found in seaweeds (4.0 mg/100 g FW) and spinach (5.1 mg/100 g FW) respectively (Isabelle et al., 2010). Perennial wall rocket (*Diplotaxis tenuifolia*) is also commonly cultivated and used as a leafy vegetable in many parts of the world (de Vos et al., 2013). Many mangrove species including *Avicennia marina* and *A. germinans* are used as food in Columbia (Leith et al., 2000). Similarly, the young shoots and leaves of *Chenopodium album* and *Amaranthus* spp. are being used as salads and vegetables, and raw fruits of *Capparis decidua* are used for pickles in different regions of India (Dagar, 2005; Rameshkumar and Eswaran, 2013).

Table 3. Halophytic plants used as traditional food and vegetables around the world.

Plant	Historical and traditional uses	Plant part	Nutritional factors	Where	Reference
Eelgrass (*Zostera marina*)	Bread; consumed by Seri Indians	Seeds / grain	Seed contains about 50 % starch, 13 % protein, and 1 % fat	South-western United States	Felger and McRoy (1975); Fleger and Moser (1976)
Palmer salt grass (*Distichilis palmeri*)	Bread; consumed as gruel (a thinner version of porridge) by Yuman and Cocopah Indians	Seeds / grain	Seed contains 79.5 % carbohydrate, 7-8 % protein, 8.4 % fiber and 1.8 % fat	Gulf of California, United States	Yensen et al. (1985); Pearlstein et al. (2012)
Pearl millet (*Pennisetum typhoidea*)	Staple food of native people	Seeds/ Grain	Gluten free and rich source of energy[a], also rich in vitamins B	Sub -Saharan Africa and northwest India	BOSTID (1990); Nambiar et al. (2011)
Quinoa (*Chenopodium quinoa*)	Soup, bread, cake, brewing beer, cooked green vegetable by native people	Seeds	Balanced in amino acids[b]	Andean highlands in Peru	Risi and Galwey (1984); Koziol (1992); Adolf et al. (2013)
Mesquite *(Prosopis glandulosa)*	Food consumed by Seri Indians	Pods and seeds	Protein content 39.9 %	Gulf of California	Fleger and Moser (1976)
Saltwort (*Batis maritima*)	Food eaten raw or cooked or pickled by the Seri Indians	Roots and stems	Essential amino acids, tocopherol, antioxidants	South-western United States	Fleger and Moser (1976); Debez et al. (2010)
Seaside purslane (*Sesuvium portulacastrum*)	Consumed as a vegetable	Leaves and stems	High values for calcium, iron, and carotene	India, Indonesia and southern China	BOSTID (1990); Lokhande et al. (2009)
Salicornia and *Sarcocornia* spp.	Salad greens, vegetable	Leaves	Good source of vitamin A, minerals, fatty acids, polyphenol	USA and European fresh markets	Ventura et al. (2011); Zerai et al. (2010)
Tetragonia tetragonioides	Frozen like spinach	Leaves	Amino acids, antioxidants,	First white settlers in Australia	Słupski et al. (2010); Ahmed and Johnson (2000)
Sea Fennel (*Crithmum maritimum*)	Food (salad) by native people; used to protect sailors from scurvy (fresh and pickled)	Leaves	High content of vitamin C	Italy; Greek islands; British Isles	Frank (1982); Ben Hamed et al. (2007)
Mountain spinach *(Atriplex hortensis)*	Leaves for salad	Leaves	High in protein	France and Central Europe	Shannon and Grieve (1999); Carlsson and Clarke (1983)
Common purslane (*Portulaca oleracea*)	Salads and soup	Fresh leaves and stem	Omega-3 fatty acids, vitamin C, vitamin A	Mediterranean region and Central Europe; Pacific Northwest of the U.S.	Shannon and Grieve (1999); Simopoulos (2004); Yazici et al. (2007)
Scurvy grass (*Cochlearia officinalis*)	As salad; used by the Dutch mariners to combat the scurvy	Fresh leaf and stem	Vitamin C, glucosinolates	Netherlands	Maat (2004); de Vos et al. (2013)

Notes: [a] The energy value of pearl millet is 361 kcal/100 g which is comparable with wheat (346 kcal/100 g) and rice (345 kcal/100 g); [b](particularly histidine and lysine) and their composition is better balanced, than in major cereals.

Table 4. The price received by producers of quinoa (a halophytic grain crop) in Bolivia and Peru (two main producing countries) compared with the cereal crops.

Crops	Country	Producer price (USD/t)										
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Quinoa	Bolivia	526.6	512.3	439.6	420.5	443.7	460.3	448.3	469.2	756.2	1320	1332.6
Rice		165.1	151.4	142.3	137.2	138	139.9	134.6	166.9	188.2	183.2	169.8
Maize		175.6	160.4	149.5	138.4	142.4	135.6	138	148.3	163.6	176.8	157.3
Wheat		190.3	180	178.4	170.1	167.3	167.8	167.2	173.6	254.2	230.6	256.4
Barley		114.8	108.7	101.1	95.8	93.5	93	92.7	98.5	145.2	148	164.3
Quinoa	Peru	335.2	339.3	318.5	319.1	299.4	352.1	360.5	390.3	547.1	1116.3	1198.6
Rice		166.2	185.4	147.9	155.2	261.6	197.3	177.2	262.3	386.4	229.2	269.5
Maize		212	226.7	224.7	211.3	202.3	232.2	250.5	299.1	386.4	425.2	
Wheat		189.1	202.5	193.4	178.2	191.5	215.5	213.9	262.3	427.4	382.1	397.2
Barley		149	165.4	153.6	140.9	142.9	157.8	168	195.1	324.9	312.3	308.5

Source: FAOSTAT (2013)

2.4. Halophytes for forages and animal feeds

Some halophytes have long been known as alternative forage and fodder crops for saline areas (Aronson, 1985; Masters et al., 2007). Seasonal feed shortages are major constraints to successful animal production in many parts of the world, and halophytic forage may be crucial in those areas (Malcolm, 1969; Aronson, 1989; El Shaer, 2010). Le Houérou (1994) reported that 100,000 ha of saline land had been planted with *Atriplex* spp. for forage production and reclamation of saline land in the Mediterranean Basin. Large scale forage production trials were conducted at the NIAB (Nuclear Institute for Agriculture and Biology), Biosaline Research Station, Lahore in Pakistan, using salt-tolerant species (Aslam et al., 1993). Projects conducted in a wide range of different climatic zones of Faisalabad, Peshawar, Bhawalpur and Karachi in Pakistan showed that saltbush (*Atriplex* spp.*)*, bluebush (*Maireana* spp.) and other halophytic plants were also highly useful additional sources of forage (Hollington et al., 2001).

Forage and seed products from halophytes can replace traditional ingredients in domestic animal diets although there are restrictions on their use due to high salt content and anti-nutritional compounds present in some species (Glenn et al., 1999; Rogers et al., 2005). The successful use of halophytes as forage for livestock production also depends on their biomass production; nutritive value and voluntary feed intake (Norman et al., 2013). Some halophytic plants, for example kikuyu grass (*Pennisetum clandestinum*), contain antimethanogenic properties that reduce methane gas production in grazing sheep and cattle and therefore offer

some hope of reducing greenhouse gas (GHG) production in ruminant livestock industries (Muscolo et al., 2013). However livestock such as sheep (Swingler et al., 1996), camels, and cattle (Khan and Ansari, 2008) thrive well on certain halophyte-based feeds. The most salt tolerant farm animals, based on the sodium chloride in drinking water, is the camel, followed by sheep and goats, whereas the least tolerant are pigs and poultry (reviewed by Masters et al., 2007). *S. bigelovii* has already been tested for seawater-irrigated agriculture in the eastern coastal region of the Arabian Peninsula, and it was shown that it can replace 25% of alfalfa in animal diets (e.g. sheep) (Abdal, 2009). Over the 84 days between weaning and slaughter, lambs fed on a halophyte-based diet (*Atriplex barclayana, Suaeda esteroa* and *S. bigelovii)* gained weight at the same rate as animals fed with conventional feeds *(e.g. Cynodon dactylon*) (Swingler et al., 1996). A feeding trial with goats and sheep using *Sporobolus virginicus* and *Distichlis spicata* compared with Rhodes grass showed that animals fed on 70% *Sporobolus or Distichlis* plus 30% Rhodes grass performed much better, in terms of the meat-fat-bone ratio and body composition, than animals fed on 100% Rhodes grass (e.g. a conventional feed) (ICBA, 2007). Similarly, an experiment conducted in Pakistan showed that the meat of animals (1-year old cow calves) fed on only halophytic grass (*Panicum turgidum*) was leaner than that of animals fed on a traditional green fodder (maize) (Khan and Ansari, 2008). In Egypt, the halophytic grasses *Leptochloa fusca, Spartina patens* and *Sporobolus virginicus* are used for forage production. Their dry matter (DM) yield ranged between 1.1 and 6.5 t DM/ ha/year making them ideal as forage crops in the desert areas where only saline water is available for irrigation (Ashour et al., 1999). Pearl millet (*Pennisetum typhoides*) can be grown as fodder also, yielding up to 6.5 t DM/ha (Jaradat, 2003). In the coastal regions of India mangrove and other halophytic species, such as *Terminalia catappa, Aeluropus lagopoides, Cynodon dactylon, and Brachiaria mutica* are used as forage for cattle, camel and goats (Dagar, 2005).

Importantly, the most productive halophytes used for forage showed a yield potential of 10 to 20 t DM/ha of biomass when irrigated with saline water (Table 5), which is equivalent to the productivity of conventional forage species under non-saline conditions (O'Leary et al., 1985; Glenn et al., 1999; Masters et al., 2007). Foxtail or buffel grass (*Cenchrus ciliaris*) which is considered a good forage for cattle, camels, and sheep in arid and semi-arid regions can be grown successfully with saline irrigation water of up to 12 dS/m (ICBA, 2011b). More than 800 different genotypes of the species have been studied by International Centre of Biosaline Agriculture (ICBA) for their adaptability to conditions in the United Arab Emirates (ICBA, 2011b). In 2011, ICBA established three model farms to produce forages from salt-

tolerant shrubs, trees and grasses using irrigation with ECw values exceeding 15 dS/m in the western region of Abu Dhabi Emirate (ICBA, 2012). ICBA has also been conducting several collaborative projects with research organisations in Saudi Arabia, Pakistan and Bangladesh for the development of biosaline fodder and forage production using *Sporobolus virginicus, Distichlis spicata*, and *Atriplex* species (*Atriplex halimus, A. nummularia* and *A. lentiformis*; ICBA, 2007). Similarly, a US-based company NyPa International has patented a cultivar (NyPa Forage^{TM}) of *D. spicata* for grain, turf and forage production. Field trials have been conducted at sites in Western Australia, South Australia and Victoria (Leake et al., 2002) using salty water as an irrigation source, and it was concluded that NyPa forage has many advantages as a plant species in constructed wetlands receiving aquaculture effluent that is high in both nutrients and salt levels (Lymbery et al., 2013).

While the above reports look highly promising, some word of caution is needed. To start with, most of the work quoted has been done by agronomists, who focus on plant biomass production rather than livestock production. Also, several important aspects should be taken into account (Norman et al., 2013). Firstly, increasing amounts of salt in the animal diet result in increased water consumption which might affect animal weight (especially in short-term experiments). So, "fat scoring" or some other indicators of animal condition should be used instead of just live-weight. Secondly, high quantities of the salt in the diet may be also long-term issue, especially for animals grazing dicotyledenous halophytes. Nearly all salt ingested by grazing animal needs to be extracted through the kidneys; this increases the energy required to metabolise the feed and the requirement for fresh water. Lastly, metabolisable energy for livestock comes from the digestion of the organic matter, not the total dry matter (O'Connell et al. 2006), and levels of metabolisable energy are generally lower in halophytes compared with other forages. This does not mean the use of halophytes as forage species has to be discarded but suggest that they should be used in a combination with a grain supplement to provide additional energy (as in ICBA, 2007; Norman et al., 2013) to ensure animal health.

Table 5. Annual biomass production (t DM/ha) of some forage halophytes under saline irrigation conditions.

Species	Irrigation water salinity (dS/m)	Biomass production (t DM/ha)	Soil type	References	Where
Atriplex lentiformis	3.5	16	Alkaline sandy prime series loam	Jordan et al. (2009)	Arizona, USA
	3.5	15-22	Alkaline sandy prime series loam	Soliz et al. (2011)	Arizona, USA
	18	18		O'Leary et al. (1985)	Arizona, USA
	13.9	9.0	Clay to clay loam	Díaz et al. (2013)	California, USA
	20	25		ICBA (2007)	ICBA, UAE
	10	14.7		Watson et al. (1987)	
A. deserrticola	18	2.2–5.5		Watson and O'Leary (1993)	
A. nummularia	10	12		Watson et al. (1987)	
A. halimus	20	14		ICBA (2007)	ICBA, UAE
Spartina gracilis	13.9	8.5	Clay to clay loam	Díaz et al. (2013)	
Spartina patens	46.9	14.4		Gallagher (1985)	Delaware, USA
Salicornia bigelovii	62.5	12- 18	Sandy	Glenn et al. (1991, 1999); Glenn (1997)	
	13.9	7.6	Clay to clay loam	Díaz et al. (2013)	California , USA
Leymus triticoides	13.1	10–13.8	Clay loam	Suyama et al. (2007)	California , USA
Festuca arundinacea	12.1	4.5	Clay loam	Suyama et al. (2007)	California , USA
Sporobulus airioides	12.4	6.7	Clay loam	Suyama et al. (2007)	California, USA
Sporobolus virginicus	30	45		ICBA (2004)	ICBA, UAE
Distichlis spicata	30	45		Masters et al. (2007)	
Allenrolfea occidentalis	13.9	17	Clay to clay loam	Díaz et al. (2013)	California, USA
Bassia hyssopifolia	13.9	3.8 - 17	Clay to clay loam	Díaz et al. (2013)	California, USA

2.5. Halophytes as oilseeds and protein crops

At least 50 species of seed-bearing halophytic plants are potential sources of edible oil and proteins. The best known are: *S. bigelovii* (Glenn et al., 1991), *Suaeda moquinii* (Weber et al., 2001), *Kosteletzkya virginica* (Gallagher, 1985; He et al., 2003), *Suaeda aralocaspica* (Wang et al., 2012), *Salvadora persica* (Rao et al., 2004; Reddy et al., 2008), *Batis maritima* (Marcone, 2003). *Crithmum maritimum* and *Zygophyllum album* (Zarrouk et al., 2003), *Nitraria sibiria, Suaeda salsa, Chenopodium glaucum* and *Descurainaia sophia* (Yajun et al., 2003). Importantly, in all cases seeds are relatively salt free although there may be significantly higher concentrations of salt in other parts of the plant (Jaradat, 2003). *S. bigelovii* has been trialled in various parts of the world such as the Middle East (Jardat, 2005; Abdal, 2009), India (Rameshkumar and Eswaran, 2013), Mexico (Gratton et al., 2008), and Africa (Zerai et al., 2010). Commercial *Salicornia* production trials have been established in the USA, Eritrea and Saudi Arabia (ICBA, 2007). *S. bigelovii* production on the desert shoreline of Mexico was higher than fresh water-grown oilseed crops such as sunflower and soybean (Fig. 2). *S. bigelovii* has shown high yields (biomass and seed) even at root zone salinities greater than 70 g/L of total dissolved salts. Under seawater irrigation (salinity 40 g NaCl/L) the seed yield was 2 t/ha (Glenn et al., 1999) which is equivalent to that of conventional crops under non-saline condition (Glenn et al., 1997). *S. bigelovii* seed has oil and protein content of 30% and 35%, respectively (Ho and Cummins, 2009) and this oil is similar in properties to safflower oil (Glenn et al., 1991; Zerai et al., 2010) (Table 6). Similarly, the perennial seashore mallow (*Kosteletzkya virginica*) produces a seed that contains 32% protein and 22% lipids (Gallagher, 1985). The fruit of *Crithmum maritimum* is rich in lipids (about 44% on a dry weight basis) with oleic acid as a major component (78.6% of the total fatty acids) (Atia et al., 2011). Seeds of *Suaeda fruticosa* could be used as a source of edible oil for human consumption because it has 74% unsaturated fatty acid (Weber et al., 2007). *Salvadora persica* is used as a source of seed oil in India and can be cultivated in soils with ECe values ranging from 25 to 65 dS/m; however seed yield declined by 40-47% at ECe values of 55- 65 dS/m compared with values of 25-35 dS/m (Rao et al., 2004). The seed of this plant contains 40-45% of oil rich in lauric (C_{12}) and myristic (C_{14}) acid widely used in cosmetic and pharmaceutical industry (Reddy et al., 2008).

Fig. 2. Productivity comparison of conventional oil seeds crops and halophytic oil seed crop (*Salicornia bigelovii*). Source: Glenn et al. (1999). The 2012 average world yield data shown for sunflower, mustard and safflower (FAOSTAT, 2013). For *Salicornia*, the reported data is from Glenn et al. (1999) for trails conducted in Mexico.

Table 6. The characteristics of oil and protein obtained from the seed of a number of halophyte species

Species	**Yield (Kg/ha)**	**Nutritional factor**	**Comment**	**Reference**
Salicornia bigelovii	2000	28% oil; 31% protein	Similar to soybean yield and quality	Glenn et al. (1991); Glenn et al. (1999)
Kosteletzkya virginica	638 - 1460	8- 25 % protein; 11.28- 15 % oil		Gallagher (1985); He et al. (2003); Liu et al. (2012)
Suaeda torreyana		25.25% oil; 89.6 % unsaturated fatty acid	Similar to the quality of canola seed oil	Weber et al. (2001)
Suaeda fruticosa		74% unsaturated fatty acid		Weber et al. (2007)
Salvadora persica		25-40 % oil, rich in industrially important lauric (C_{12}) and myrestic acid (C_{14})	Industrial oil; can be used in soap and detergent making	Reddy et al. (2008)

2.6. Halophytes as energy crops (biofuels and fuelwood)

Halophytes can be also a highly valuable source of fuels such as bioethanol, biodiesel and fuelwood. Globally, nearly 1.3 billion people live without access to electricity, and 2.6 billion lack clean cooking facilities, mostly in the developing countries of Asia and Africa (International Energy Agency, 2012). These people often rely on the wood obtained from various plant species in their normal environments. In addition, it is predicted that world petroleum reserves will be substantially delpeted by the middle of this century if used at the current rate, while coal reserves may run out over the next 100 years (Shafiee and Topal, 2009). Consequently, the use of bioethanol as a renewable energy source has been steadily increasing (Demirabas et al., 2011; Abideen et al., 2012) and is regarded as a viable alternative substitute for gasoline in the transportation sector (Del Campo et al., 2006). The global production of biofuel has grown from 16 billion litres in 2000 to 100 billion litres in 2011, currently accounting for 3% of the total road transport fuel globally (International Energy Agency, 2012). In Brazil, approximately 20% of all car fuel is obtained from bioethanol produced from sugarcane (Eshel et al., 2010). However, demand is leading to increased competition between crop production for food and biofuel, as currently biofuels are produced from conventional crops such as sugarcane, soybean, and corn using prime land and fresh water (Del Campo et al., 2006; Abideen et al., 2011). This has already had a major impact on food prices and led to scepticism about the sustainability of the bioethanol industry (Abideen et al., 2011). Thus, the need for alternative sources for biofuel production is crucial.

For socially acceptable and economically sustainable biofuel production, the industry should co-exist with the food industry but not compete with or replace it. Halophytes that are capable of growing in harsh environments, that are not suitable for conventional crop production, may be ideally suited for this role, alleviating the economic and ethical pressure on food production (Eshel et al., 2010). Halophytes can be grown without competing with food crops for good quality soil and water resources (Rozema and Flowers, 2008; Qadir et al., 2008) and can exploit saline lands such as coastal zones for this purpose (Liu et al., 2012). Indeed, salt tolerant plants are often used to provide fuel for cooking and heating in developing countries (Ladeiro, 2012). Biofuel from the halophytic lignocellulosic biomass (i.e. plants dry matter which is composed of cellulose, hemicelluloses and lignin) will be an environmentally sound and sustainable alternative to address the food versus fuel production debate (Abideen et al., 2012). In addition, this approach might ease the pressure on the petroleum fuel industry and help to solve the issue of CO_2 emissions currently facing the

aviation industry (Hendricks et al., 2011). Thus growing halophytic plants as the energy feedstock will not only provide relief for the world's dependence on fossil fuel, but will also help to reduce the issue of global warming by reducing production of greenhouse gas.

Several studies have shown that various salt-tolerant plant species have potential to produce biomass and renewable energy from salt-affected environments (Qadir et al., 2008; Eshel et al., 2010; Liu et al., 2012). A number of species including the halophytes *Tamarix chinensis, Phragmites australis, Miscanthus* spp. and *Spartina alterniflora* were evaluated as biofuel crops for ethanol production in the coastal zone of China (Liu et al., 2012). Halophytic perennial grasses grown in the coastal region of Pakistan (*Halopyrum mucronatum, Desmostachya bipinnata, Phragmites karka, Typha domingensis* and *Panicum turgidum)* were also shown to be highly suitable for bio-ethanol production (Abideen et al., 2011). The halophytic grass *Panicum virgatum* has been shown to produce similar yields of ethanol to corn (*Zea mays*) which is grown extensively as a conventional food crop as well as for ethanol production (Hendricks and Bushnell, 2008; Liu et al. 2012) (Table 7). Other halophytic species belonging to the genera such as *Prosopis* and *Tamarix* are also highly suitable for fuel wood production. In addition, sugar beet (*Beta vulgaris*), nipa palm (*Nypa fruticans*) and Kallar grass (*Leptochloa fusca)* are salt-tolerant plants that were identified sources of liquid and gaseous fuel (Jaradat, 2003). The perennial halophytic *Tamarix* species can survive under conditions of extreme salinity, drought and flood and are capable of producing more biomass for bioethanol production than most other conventional shrubs grown in the same environment. Experiments conducted in different parts of the world showed that biomass yield from *Tamarix spp.* can range between 19.5 (Liu et al., 2012) and 52 t/ha/year (Eshel et al., 2010), and the heat value of one ton of *T. chinensis* fuel wood is equivalent to that provided by 0.7 ton of standard coal (Liu et al., 2012). In Mexico, Global Seawater Inc. is growing *S. bigelovii* for biodiesel production at farms in Bahia Kino and Tastiota. Between 850 and 950 litres of biodiesel can be produced from S*alicornia* plants cultivated on one hectare of coastal land (Christiansen, 2008). On the basis of these results, the company was planning to expand its cultivation to 5,000 ha for commercial production in the state of Sonora, Mexico. Recently, with the support of Boeing, Etihad Airways and UOP Honeywell, the Masdar Institute of Science and Technology has started sea water irrigated S*alicornia* cultivation in the Abu Dhabi region to produce bio-fuel for the aviation industry (ICBA, 2011a): Etihad Airways conducted a successful demonstration flight by using biofuel refined in U.A.E. (http://boeing.mediaroom.com/index.php?s=20295&item=128951).

Table 7. Comparison of ethanol production from a conventional food crop, *Zea mays,* and a halophytic grass, *Panicum virgatum*

Feed stock	Crop yield (t/ha)	Ethanol production	
		(L/t)	(L/ha)
Corn (*Zea mays)*	6.75	300-340	2000-2300
Switch grass (*Panicum virgatum)*	12.50	280-380	3500-4750

Sources: Adapted and modified from Hendricks and Bushnell (2008), and Liu et al. (2012).

2.7. Halophytes for phytoremediation

2.7.1. Desalination

Halophytic plants are capable of accumulating high concentrations of NaCl in their tissues (e.g. up to 39% in a saltbush; Barrett-Lennard, 2002). Assuming this capacity can be matched by high biomass production, halophytic species can be a biological solution to rehabilitate saline-sodic or salt-affected land by extracting significant amounts of salt from the soil, establish plant cover, and lowering the water table.. The potential of some halophyte species for the purpose of desalination is summarised in Tables 8 & 9. A marked decline in soil EC has been measured during experiments, both in the field and glasshouse (Table 8), equating to the removal of between 1 and 6 tonnes of NaCl per hectare per year (depending on the actual initial salinity level in the soil). These numbers are in a good agreement with estimates for potential NaCl removal from the soil calculated on the basis of Na^+ and Cl^- concentration in dry matter and overall biomass production under saline irrigation (Table 9). Here, the estimated NaCl removal ranged between 2 and 6 tonnes of salt per hectare per year. It should be commented though that the use of halophytes as a desalinization tool cannot be guaranteed in all cases, as their productivity may be significantly affected not only by salinity but also by other environmental conditions (Shabala, 2013). For example, the annual biomass yield of *Atriplex* species in the Western Australian low rainfall zone (330-370 mm/year) was only 0.4 to 0.7 t DM/ha (Norman et al., 2008) compared with 15-18 t DM/ha under irrigated conditions (O'Leary et al., 1985; Pasternak et al., 1985; Soliz et al., 2011). Thus, their suitability for the phytoremediation process in those climatic conditions (i.e. low rainfall and non-irrigated) is highly questionable. In addition, some of the salt accumulated in the plant parts can be recycled back to the soil in the form of leaf litter (Barrett-Lennard, 2002). Also, some of the numbers reported in Tables 8 and 9 are either predicted values extrapolating results of glasshouse experiments to the field, or are published by the same authors.

Table 8. Salt removal from the soil as reported for some selected halophyte plants.

Plant species	EC change, dS/m		Experimental system	Trial duration	Reported Na^+ removal, t/ha (per test period)	Estimated overall NaCl removal, t /ha/year	Reference
	From	To					
Arthrocnemum indicum	75	23	Field	1 year	1.65[b]	4.19	Rabhi et al. (2009)
Excoecarcia agalloha	4.9	2.4	Field	120 days	0.16	1.12	Ravindran et al. (2007)
Ipomoea pes-caprae	4.9	2.6	Field	120 days	0.15	1.08	Ravindran et al. (2007)
Sesuvium portulacastrum	4.9	2.5	Field	120 days	0.18	1.42	Ravindran et al. (2007)
Suaeda maritima	4.9	1.4	Field	120 days	0.20	1.51	Ravindran et al. (2007)
Suaeda fruticosa	57	14.8	Field	180 days	0.22	0.66	Zorrig et al. (2012)
Suaeda salsa	16.2[a]	13.5[a]	Field	120 days	1.92	4.87	Zhao (1991)
Tecticornia indica	57	11.8	Field	180 days	0.75	3.53	Zorrig et al. (2012)
Arthrocnemum indicum	19	10.1	Pots	170 days	0.71	1.80	Rabhi et al. (2009)
Sesuvium portulacastrum	19	9.1	Pots	170 days	2.5	6.35	Rabhi et al. (2009)
Suaeda fruticosa	19	12.0	Pots	170 days	0.8	2.00	Rabhi et al. (2009)

Notes: [a] Calculated based on reported soil Na content (reported in g kg^{-1} DW in original article); [b] Calculated assuming the size of the "large tuft" being 4 m^2 (the original paper reports Na^+ removal in mmol Na^+ per tuft without indication of the tuft size).

Table 9. Estimated phyto-desalinisation potential of some selected halophyte species.

Plant species	Biomass, t DW/ha/year	Experimental system	NaCl content in irrigation water, mmol	Shoot Na^+ content, % DW	Shoot Cl content, % DW	Estimated NaCl removal, t/ha/year	Reference
Atriplex nummularia	28.9	Field	75	9.1	12.8	6.32	Pasternak et al. (1985)
Atriplex nummularia	15.3	Field	510	12.2	18.3	3.75	Pasternak et al. (1985)
Atriplex lentiformis	17.9	Field	680	6.8	8.8[1]	2.79	O'Leary et al. (1985)
Atriplex lentiformis	4.2	Field	30	8.3	11.0	0.81	Glenn et al. (2009)
Atriplex canescens	17.2	Field	680	8.6	11.2[1]	3.41	O'Leary et al. (1985); Glenn and O'Leary (1985)
Batis maritima	17.4	Field	680	12.0	15.6[a]	4.80	O'Leary et al. (1985); Glenn and O'Leary (1985)
Salicormia europaea	15.4	Field	680	13.9	18.0[1]	4.91	O'Leary et al. (1985)
Kalidium folium	8.7	Pots	400	16.8	15.3	2.79	Zhao et al. (2005)
Suaeda salsa	7.7	Pots	400	15.5	14.3	2.06	Zhao et al. (2005)

Notes: [a] Chloride content was not measured in this work and was estimated as being 1.3 of those for sodium.

In Western Australia, NyPa grass of the genus *Distichlis* lowered the saline groundwater table by about 0.25 meters after a year of cultivation (Leake et al., 2002). Similarly, Barrett-Lennard (2002) suggested that high biomass producing saltbushes (*Atriplex* species) can transpire water to lower watertables and Barrett-Lennard and Malcolm (1999) who estimate that such stands of saltbushes can use ~60-100 mm of groundwater over two years (based on increases in the concentration of chloride in the root-zone).

The agricultural practices used also need to be taken into account. Tillage of soil will increase the leaching of salt from surface soils into the subsoil, and plant density may also have an impact on the balance between capillarity and leaching events, as the shadows cast by plants on the soil surface will increase the depth of boundary air layers by influencing soil temperature, decreasing evaporation from the soil surface. Furthermore, any net reduction in salinity will depend not only on salt removal, but also on whether or not there is continuing salt input to the soil profile - from, for example, saline groundwater. Hence, a more robust evaluation of the suitability of halophytes for the practical purposes of land desalination is needed.

2.7.2. Phytoremediation of polluted soils

Rapid industrialization has triggered significant production and a consequent accumulation of heavy metals such as Cd, Cu, Pb and Zn in the soils of tidal marshes (Flowers et al., 2010; Govindasamy et al., 2011). Arsenic was found to be a major by-product of pesticides and wood preservatives; Cd from paints and pigments; Cr from tanneries and steel industries; Pb from battery manufacture, herbicides and petroleum production; and Hg from medical waste (Ali et al., 2013; Wuana and Okieimen, 2011). These accumulated elements in soil and water bodies can cause a risk to humans and other living organisms (Khan et al., 2010). A clean-up of these contaminated soils is needed but challenging, especially in terms of the cost, when conventional remediation tools such as soil incineration or washing are used (Sheoran et al., 2011; Wuana and Okieimen, 2011). The use of hyper-accumulating plants may reduce this cost dramatically. Some halophytic plants (see Table 10) show an ability to grow in those polluted areas and are widely advocated for phytoremediation purposes such as phytoextraction or phytostabalization (Lutts et al., 2004; Manousaki and Kalogerakis, 2011). Phytoextraction refers to the process of removal of a contaminant (e.g., Ag, Cd, Co, Mo, Pb, Zn) from the soil by hyperaccumulating plants. During this process plant roots take up contaminants from the soil and store them in their aboveground parts. This can be followed by harvesting and disposal of the plant biomass. Seagrasses and salt marsh plants are good examples of species that can extract heavy metals from sediments and accumulate them in their biomass (Cambrolle et al., 2008; Lewis and Devereux, 2009). Similar results have been observed using halophyte species such as *Sporobolus virginicus* (Eid and Eisa,

2010), *Atriplex halimus* and *Tamarix smyrnensis* (Manousaki and Kalogerakis, 2011). It has been reported that *Atriplex halimus* can remove 4.15 kg of Cd and 2.2 kg of Zn /ha/year respectively assuming plant yield is maintained at 5 t DM/ha/year (Lutts et al., 2004).

Table 10. Selected examples of heavy metal accumulation (mg/kg DM) by halophyte plants.

Plant species	Element	Amount accumulated (mg/kg DM)	References
Sesuvium portulacastrum	As	155	Lokhande et al. (2011)
Atriplex halimus	Cd	830	Lutts et al. (2004)
Atriplex halimus	Zn	440	Lutts et al. (2004)
Atriplex species	B	192	Watson et al. (1994)
Sesuvium portulacastrum	Cd	350-700	Ghnaya et al. (2005)

Lokhande et al. (2011) investigated *Sesuvium portulacastrum* as a potential candidate to reclaim arsenic (As) affected land as it showed good plant growth, and a capacity to accumulate up to 155 µg/g dry weight of As. However, most of the known hyper-accumulators are slow growing, which limits their capacity to remove contaminants from the soil. Also, the efficiency of phytoextraction depends strongly on soil properties and, thus, the bioavialibility of the heavy metals in the soil (Ali et al., 2013), in addition to their ability to tolerate heavy metal accumulation. In this context, phytostabilization, e.g. the ability of vegetation to prevent the dispersal of a contaminant from the affected site or to contain the contaminant *in situ*, may be a more promising way to use such halophytes.

2.8. Halophytes as medicinal plants and other commercial products

Halophytic plants can be grown not only for food and fuel but also for medicinal purposes. In traditional medicine some species play an important role in treating diseases in different parts of the world. For example, *Ipomoea pes-caprae* is used to treat fatigue, strain, arthritis, rheumatism and menorrhagia (Rameshkumar and Eswaran, 2013). *Ipomoea* species contain many biologically active constituents such as alkaloids, phenolics, coumarins, flavonoids etc. and these plants show analgesic, antimicrobial, and anticoagulant properties (reviewed by Meira et al., 2012). *Terminalia catappa* leaf is used to treat liver disease in Taiwan (Rameshkumar and Eswaran, 2013) and in India it is used as a cardiac stimulant, for treating dermatosis and hepatitis, and has shown better antibacterial activity than some commercially used antibacterial agents (Chanda et al., 2011). *Mesembryanthemum crystallinum* is used for its polyol-rich food which is known to have disease-preventing functions (Agarie et al., 2009). In addition to being used as a fuel wood, *Tamarix gallica* has bioactive compounds such as phenols, glycosides, tannins,

flavonoids, steroids, saponins, and alkaloids and is often used for treatment of liver disease in traditional medicine (Ksouri et al., 2009). The phenolic compound from this species is known for its anti-aging, anti-inflammatory and anti-carcinogenic properties (Mandloi et al., 2013). The facultative halophyte *Crithmum maritimum*, also known as crest marine or marine fennel, has been largely used for nutritional and medicinal purposes as it is rich in several bioactive substances, minerals, vitamin C, essential oils and other biomolecules (Atia et al., 2009; Meot-Duros et al., 2010). Oils extracted from this plant showed the presence of high concentrations of fatty acids of the ω-3 and ω-6 series which have beneficial effects against coronary heart diseases (Guil-Guerrero and Rodriguez-Garcia, 1999).

Halophytes also have a range of other uses. For example, screwpine (*Pandanus fascicularis*) are rich in methyl ether of □-phenylethyl alcohol (65-80%) and are used as perfume and flavouring ingredients (Dutta et al., 1987). A perennial desert shrub guayule (*Parthenium argentatum*) is a source of natural rubber that can be grown under saline conditions of up to 7.5 dS/m (Hoffman et al., 1988). Halophytic grass species such as seashore paspalum (*Paspalum vaginatum*) and marine couch (*Sporobolus virginicas*) are used commercially for turf production for golf and landscape development (Depew and Tilman, 2006). *Suaeda monoica*, a succulent chenopod, is used in the paper industry in India (Rameshkumar and Eswaran, 2013). Many halophytic plants have potential for saline floriculture production where salinity levels are in the range of 5-15 dS/m. In Israel, the blue bush (*Maireana sedifolia*) is used for its cut branches and is commonly exported to Europe (Cassaniti et al., 2013).

2.9. Conclusion

This paper has reviewed the current status of halophytes for their potential use as a source of food for humans and livestock, as crops for biofuel,for medicinal and other industrial purposes, as well as their capacity to rehabilitate salt-affected land. Crop and forage species currently used in agriculture are a result of many centuries of domestication and being selected for a particular set of generally favourable environmental conditions; furthermore most of them are not able to survive or grow in highly saline situations. At the same time, the need to expand agricultural production into previously non-arable land to meet the 2050 challenge of feeding over 9 billion people requires immediate and drastic action. One of the most obvious solutions is try to make an "inventory" of plant species suitable for human or animal use that can be successfully cultivated in harsh saline environments. Halophytes are a diverse group of plants with significant potential to meet this challenge and can be successfully incorporated into future farming systems. The latter, however, will depend not only on the plant's ability to deal with

high levels of NaCl in the soil but also on the farmers' acceptance of new practices, crop nutritional quality, adequate levels of biomass production, market development and price, overall cost-benefit of production as well as government policies. However, there is little doubt that the time has come for the use of halophytes in saline agriculture to move from the laboratory and small field trials to large-scale commercial production. .

Chapter 3

Growth responses of *Atriplex lentiformis* and *Medicago arborea* in three soil types treated with saline water irrigation*

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Abstract

The ready availability of fresh water for irrigation of fodder crops is decreasing and costs are increasing due to competing demands for fresh water from industrial and other agricultural uses and, more broadly, due to rising global population pressures. At the same time, large amounts of industrial wastewater, often of high in salt content, are produced by urban activities or industries which require reuse or disposal. So it is valuable to investigate the use of these saline industrial wastewaters for agriculture to ease the pressure on freshwater supply for irrigation. The purpose of this study was to investigate the effect of saline water irrigation on the performance of two plant species, *Atriplex lentiformis* (xero halophyte) and *Medicago arborea* (glycophyte). Plants were grown under glasshouse conditions in three soil texture setups; 1) clay, 2) sandy loam and 3) sandy loam over clay (texture-contrast). These were irrigated with 0.04 (control), 8 and 16 dS/m saline waters. Both plant species yielded higher biomass in the clay texture compared to other soil texture setups under all irrigation treatments. When water salinity increased progressively from 0.04, 8 to 16 dS/m the total dry biomass yield of *A. lentiformis* was increased by 1.4, 1.3 and 1.5 times in clay, texture-contrast and sandy loam soil respectively but it was significantly reduced in *M. arborea* by 1.5, 3.1 and 5 times in the same soil set-ups. There was no significant variation in chlorophyll fluorescence with salt treatments but stomatal conductance was affected by salinity in *M. arborea*. The reduction in the stomatal conductance in *M. arborea* at 8 and 16 dS/m salinity was 41% and 74% of control, respectively. Overall, leaf ion content (Na^+ and Cl^-) also increased with increasing salinity treatment in both plants, but significant effects were seen only in sandy loam soil for both species. The leaf sap Na^+ increased

by 1.5 – 2 fold compared with control in *A. lentiformis* whereas a 6 and 9 fold increase was reported for *M. arborea* grown at 8 dS/m and 16 dS/m salinity levels, respectively. Our results indicated that both osmotic effect and specific ionic toxicity impacted physiological performance in *M. arborea* while *A. lentiformis* plants were insensitive to both components of salt stress. Plant performance in the sandy soil was not as good as in clay, indicating that soil texture and structure may have a significant role in the salt stress process under saline irrigation. In the clay texture plants may be benefiting from the naturally inherited high nutrient status compared to the sandy textured soil. That could be one of the reasons for high biomass yield (leaf and stem) in clay regardless of the salinity treatments.

Key words: salinity; Atriplex; Medicago; forage species; biomass yield; saline irrigation; Soil texture; saline agriculture

3.1. Introduction

Global food production is strongly related to the available land area and water resources and other input resources available. Clearly it needs to match the burgeoning human population without degrading the resources used to produce it. The world population has increased from 5.7 billion in 1994 to 7.2 billion in 2014 and it is projected to reach 8.1 billion in 2025 and 9.6 billion in 2050 (United Nations, 2014). This increase will increase food demand by as much as 70% by 2050 (Bruinsma, 2009). At the global level, 70% of all available freshwater is already used for irrigated agriculture, and in under-developed countries this exceeds 90% (FAO, 2011). This indicates that freshwater resources are already overstretched and cannot sustain demand from the irrigated agriculture without major increases in efficiency. Due to the high demand and competition for freshwater, from both urban and industrial users, alternative sources of irrigation water are needed along with selected plant species and increases in water use efficiency. There are various sources of low quality water, i.e. saline water, that can be used for irrigation purpose, such as water coming from natural or secondary salinized aquifers (Ayers and Schoneman, 2006), subsurface drainage from irrigated fields (Grattan et al., 2004), industrials sources (Gerhart et al., 2006) and brine from desalinisation plants (Jordan et al., 2009). But most conventional crops cannot tolerate very saline environments and their production under these conditions may be economically unsustainable. It has been estimated that global salt induced land degradation and resulting production losses in irrigated areas could be as high as US$27.3 billion per year (Qadir et al., 2014). One alternative is to use saline waters and land for the

production of halophytic crops. There is an increasing number of halophytes which have been tested and used for food, fodder and fuel production purposes (Panta et al., 2014; 2015).

Atriplex lentiformis is a native widespread halophytic shrub of south-western united States and northern Mexico and it has been used for various purposes such as forages, re-vegetation and rangeland enhancement programs around the world (Gupta and Arya, 1995; Brownings et al., 2006) and as use as an irrigated forage (Watson et al., 1987; Bauder *et al.*, 2008; Glenn et al., 2009; Jordan et al., 2009). The productivity of *A. lentiformis*, however, can vary dramatically depending on irrigation regimes and soil types (e.g 1 – 20 t/ha). For example, in USA *A. lentiformis* produced 12 to 20 tonnes dry matter per hectare per year under saline water irrigation (Watson et al., 1987). Similar numbers were reported for a field trial in the Coastal Sonort Desert, where it produced 18 t/ha/year of dry biomass under saline water (40,000 mg/L) irrigation (Glenn and O'Leary, 1985). Research conducted in Pakistan showed that *A. lentiformis* produced up to 8 tonnes dry matter/ha/year under rainfed condition (Mahmood et al., 1993). However, in Western Australia, the productivity of the *Atriplex* plantations was very low and did not exceed 1 t/ ha /year in saline texture-contrast soils under rainfed conditions (Barrett-Lennard et al., 1990). So the causal link between soil types, irrigation schedule and concentration of salt in the irrigation water has to be established to optimize *Atriplex* production. *Medicago arborea*, a salt-tolerant glycophyte, is a dense, leafy, woody shrub with a maximum height of 2 to 4 m under favorable conditions (Dear et al., 2003). This plant is native to coastal areas of Mediterranean regions and has been cultivated as a forage crops since Roman times (Lefi et al., 2012). It is adapted very well to infertile and rocky soil and shows a high potential as a valuable fodder for low rainfall climatic conditions with alkaline soil (Dear et al., 2003). It is popular for its high nutritive values and preference given by small ruminants (Lambert et al., 1989; Amato et al., 2004). However, a concentration of 100 mM NaCl significantly reduced the plant growth in *M. arborea* (Sibole et al., 2003). Boughalleb et al. (2009a,b) suggested that *M. arborea* can grow well only below 100 mM NaCl in the soil; beyond this concentration, salt toxicity restricted plant growth. It remains to be shown to what extent *M. arborea* can be used for saline agriculture purposes, and how its production is affected by soil types and amounts of irrigation water.

High irrigation rates can cause waterlogging problems in clayey textured soils, and it is estimated that 10 % of global land is adversely affected by waterlogging (Setter and Waters, 2003). Plant performance is not only affected by the soil and/or water salinity, but waterlogging also considered a major factor (Shabala, 2011). Plants under saline and waterlogged conditions may have to face a double-stress to growth. The major constraints are depleted oxygen and

associated hypoxia leading to internal energy stresses (Bailey-Serres and Voesenek, 2008) and inability to maintain membrane potential (Shabala et al., 2014). Waterlogging also causes reduction in soil redox potential, and increased production of toxic compounds by plant roots (Shabala, 2011; Zeng et al., 2013). As a result of these changes, plant stomatal conductance and leaf water potentials will decrease and plant shoot and root growth will reduce and ultimately the plant may die (Barrett-Lennard, 2003).

Most previous studies have been conducted with only a single soil texture or irrigation regime. Given the speculation of possible difference in plant performance based on the growing condition, it was necessary to compare plant responses to salinity in a range of different soil textures and irrigation regimes. Therefore, the aim of this work was to compare the phenotypic and physiological responses of *Atriplex lentiformis* and *Medicago arborea* to different levels of irrigation salinity in clay, sandy loam and texture-contrast (sandy loam/clay) soil under two irrigation levels.

3.2. Material and methods

3.2.1. Plant material and experimental setup

Atriplex lentiformis and *Medicago arborea* seeds were obtained from the AustraHort Seed Merchant, Queensland. Seedlings were grown in a glasshouse at the Horticultural Research Centre at the University of Tasmania in Hobart, Australia in 2012. Two-month old seedling of *A. lentiformis* and *M. arborea* were transplanted into 30 cm tall five litres plastic pots having one of the following: (1) sandy loam, (2) clay soil and (3) texture-contrast (sandy loam/clay) soil. The nutrient properties of this soil are presented in Table 1. A nappy liner was put at the base of each pot to prevent the loss of soil materials during drainage. The texture-contrast soil profile was artificially made by filling the lower half of the pot with clay soil and the upper half with sandy loam soil. After 3 weeks of transplanting, salinity treatments were started. Plants were irrigated with three levels of saline water (0.04 dS/m, 8 dS/m, 16 dS/m) prepared by adding appropriate amount of NaCl to the tap water. Irrigation was given at the rate of 700 mm/year and 1400 mm/year rate (hereafter, it is described as ‘low’ and ‘high’ irrigation rate respectively). Irrigation water was applied twice a day. Total water applied per pot/day was 340 ml in low irrigation and 680 ml in high irrigation regimes. The pots were arranged in a randomized complete block design with five replicates per species. After treatment initiation, plants were allowed to grow for five months and then harvested for biomass measurement.

Table 1. Initial selected soil chemical and nutritional properties of the soil used for the experiments. Clay soil used for this treatment was reactive clay from dolerite colluvium on the university farm and sandy loam was obtained from Males Sand Pit, South Arm Road, Tasmania.

Particulars	Clay soil	Sandy loam soil
Electrical conductivity saturated paste extract (dS/m)	1.02	0.41
pH (soil:water 1:5 ratio)	6.5	5.6
Total organic carbon (%)	1.98	1.36
Nitrate nitrogen (mg/kg)	95	23
Phosphorus Colwell (mg/kg)	36	17
Potassium Colwell (mg/kg)	230	98
Exchangeable ions (cmol(+))/kg		
Ca^{2+}	8.67	6.34
Mg^{2+}	3.21	2.23
Na^{+}	1.10	0.51
K^{+}	0.44	0.20

3.2.2. Photosynthetic and gas exchange parameters

Measurements of stomatal conductance were carried out on sunny days from the adaxial surface (upper surface) of the youngest fully developed leaf under glasshouse conditions. The measurements were taken in well watered plants by using a leaf porometer (model SC-1, Decagon Devices, Inc. USA) between 9 to 11 am and 1 to 3 pm. After that chlorophyll content in the leaf was measured by chlorophyll meter SPAD 520 (Konika, Minolta, Sensing, Inc. Japan). The maximum photochemical efficiency of photosystem II (PSII; chlorophyll fluorescence Fv/Fm ratio) was recorded from the upper surface of the fully developed leaves by using a hand held chlorophyll fluorometer (OS-30P, Opti-Science, Inc. USA) in dark adapted plants as described in Smethurst and Shabala (2003) .

3.2.3. Osmolality and leaf ion content

The youngest fully-expanded leaf was harvested and placed into Eppendorf tubes and stored in a freezer. The frozen leaf was defrosted and the sap was extracted by hand-squeezing the leaf samples as described in Cuin et al. (2009). The extracted leaf sap was centrifuged at 2000 RPM for 10 min to remove solid residues. Approximately 20 µl of the supernatant was used to measure sap osmolality with a vapour pressure osmometer (Vapro; WescorInc, Logan, UT, USA). An additional 50 µl of the collected supernatant was diluted with 5 ml of distilled water and used for the determination of Na^{+} and K^{+} concentration (in mM) using a flame photometer (Corning 410C, Essex, UK). Five replicates for each treatment for both plant species were assessed. The Cl^{-} was quantified from the remaining diluted supernatant by microelectrode

ion flux measurements (the MIFE™ technique). Briefly, chloride concentration was measured using the ion-selective microelectrode technique available in our laboratory (MIFE, University of Tasmania, Australia). Commercially available Cl^- ion-selective cocktail was used (liquid ion exchanger, LIX, selective for Cl^-, catalogue No. 24902, Sigma-Aldrich, St. Luis, MO, USA). The principles of the MIFE measurements are available in previous publications (Shabala et al. 1997; Shabala et al. 2006). The microelectrodes were uniformly pulled from non-filamentous borosilicate glass capillaries (GC 150-10, Harvard apparatus Ltd, Kent, UK) using a vertical puller (PP-830, Narishige, Tokyo, Japan) followed by drying overnight in an oven at 225 °C and salinized with tributylchlorosilane (Sigma, catalogue no. 282707). For further use, electrode tips were broken to achieve external tip diameters of 2–3 µm by moving electrode blanks against a flat glass surface using a micromanipulator. Electrodes were then backfilled with 0.5 M KCl followed by front-filling with Cl^- ion-selective cocktail (LIX). The prepared Cl^- electrode was mounted on a 3D-micromanipulator (MMT-5, Narishige) and calibrated in a set of three standards encompassing measured ranges of Cl^- ions using a three-point calibration (ranging from 0.5 to 180 mM). Electrodes with a Nernst slope of less than 50 mV per decade and/or correlation less than 0.999 were discarded from measurements. A standard non-polarising Ag/AgCl reference electrode was prepared by inserting a silver wire into a capillary containing 1% agar prepared in 1 M KCl. Then, a chloride sensitive microelectrode and the reference electrode were placed into a beaker containing a sample solution and voltage values were recorded by the MIFE CHART software. The samples were measured for 5-10 minutes or until a steady reading of ion flux were observed on the computer screen. The recorded values in mV were converted into concentration values using the calibrated Nernst slope of the electrode using the MIFEFLUX software (Shabala et al., 1997; Newman, 2001; Shabala et al., 2006; Shabala et al., 2012). The data was stored in ASCII format on a personal computer and transferred to Excel for data analysis.

3.2.4. Biomass

At harvest, plant leaves and stems were separated and fresh weights were measured. Plants were dried at 70 °C for 72 h and dry weights were recorded.

3.2.5. Data analysis

Data were analysed by analysis of variance using Proc GLM in SAS 9.2 for the overall test and means. Pairwise differences were compared using the least significant difference method at the 5% level of probability. The statistical significance of correlations between data

sets was calculated using Pearson's r values. Different low-case letters in each panel of the figures indicate significance at $p < 0.05$.

3.3. Results

3.3.1. Leaf biomass (fresh weight and dry weight)

Both the amount of salt in the irrigation water and soil type had a significant impact on plant performance. Salinity levels up to 16 dS/m did have a strong positive influence on plant performance in *A. lentiformis* (Fig. 1) but there was a negative effect for *M. arborea* (Fig. 2). Plant fresh leaf weight showed, on average, a positive effect of quantity and quality of irrigation water on the growth of *Atriplex lentiformis* in all treatments. The fresh leaf biomass yield of *Atriplex lentiformis* varied by 3.5 times among the treatments, ranging from 24 g/plant to 84 g/plant (Fig. 3). The yield increased significantly ($p<0.05$) when the irrigation salinity levels increased from 0.04 ds/m to 8 dS/m. The t-test (LSD) shows a significant effect ($p<0.001$) of soil types and salt levels on the fresh leaf biomass yield. The mean fresh leaf yield of plants grown in sandy loam, texture-contrast and clay soil were 45.5 g, 54.1 g, 75.5 g, respectively (differences significant at $p<0.05$; data not shown).

Fig.1 *Atriplex lentiformis* grown in different saline treatments in clay soil.

Fig. 2. *Medicago arborea* grown in different saline treatments in clay soil.

Thus, salinity level up to 16 dS/m appeared to be beneficial for the growth of *Atriplex lentiformis.* The leaf fresh weight (LFW) and leaf dry weight (LDW; Fig 4 A and B) yield varied significantly ($p<0.001$) based on the soil types and water salinity levels but not to the irrigation level. The yields (LFW and LDW) were higher in clay soil compared to sandy loam soil. The mean LDW yield of the plant grown in sandy, duplex (texture-contrast) and clay soil were 10.0 g, 10.4 g and 16.8 g/ plant respectively.

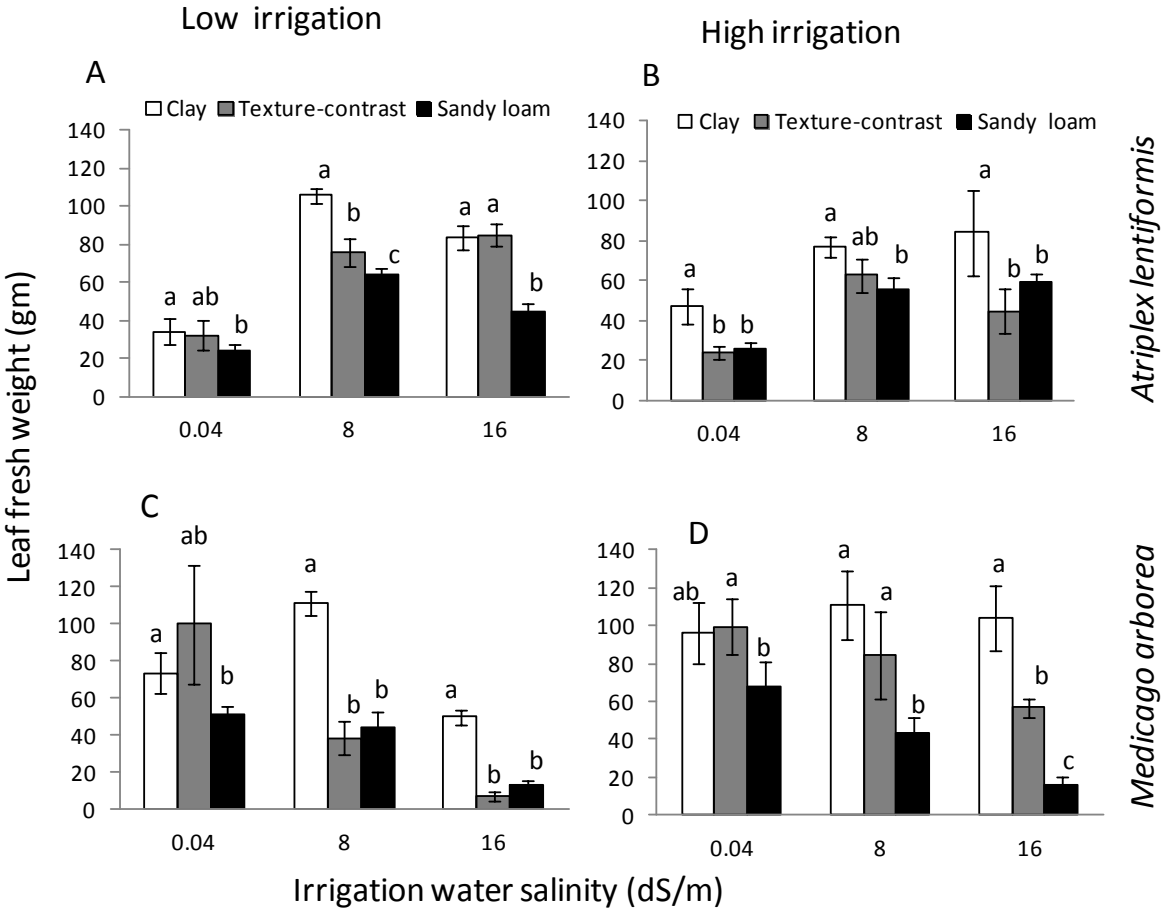

Fig. 3. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. fresh weight of leaf of *Atriplex lentiformis* (A, B*)* and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

In *M. arborea*, the LFW and LDW were significantly decreased in the texture-contrast and sandy loam soil at 16 dS/m compared to the clay soil. Interestingly, both LFW and LDW were higher in clay soil at 8 dS/m salinity from both low and high irrigation levels in all salinity treatments (Fig. 3 and 4). Overall, soil types and salinity levels show a significant ($p<0.001$) effect on leaf yield.

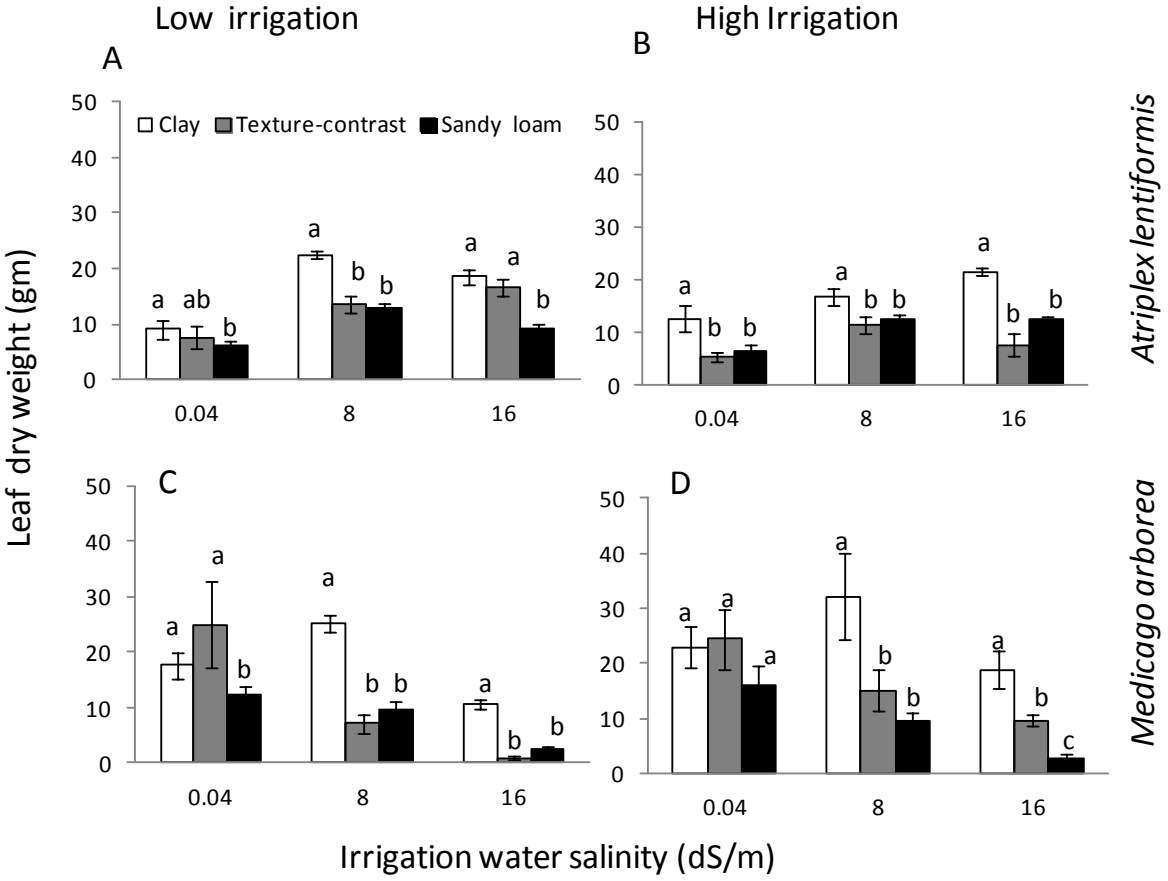

Fig. 4. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. dry weight of leaf of *Atriplex lentiformis* (A, B) and *Medicago arborea* (C,D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

3.3.2. Stem fresh weight and dry weight

The salt levels and soil types have significant effect (< 0.0001) on stem fresh weight (SFW) and stem dry weight (SDW) of both *Atriplex lentiformis* and *Medicago arborea*. However, irrigation level has no significant effect on plant stem weight. The plants grown in the clay soil had higher SFW and SDW than that of either the other texture situations. The SFW and SDW of both *Atriplex lentiformis* and *Medicago arborea* were significantly increased in the clay soil compared to control treatment at 8 dS/m, but this trend did not occur in the other texture situations (Fig. 5 and 6). Both SFW and SDW were significantly ($p<0.001$) decreased in *Medicago arborea* at 16 dS/m in all soil types, though and the effect was more pronounced in sandy loam. Meanwhile, in *Atriplex lentiformis* SFW and SDW were unaffected in both the sandy loam and the texture-contrast textures up to 16 dS/m water salinity.

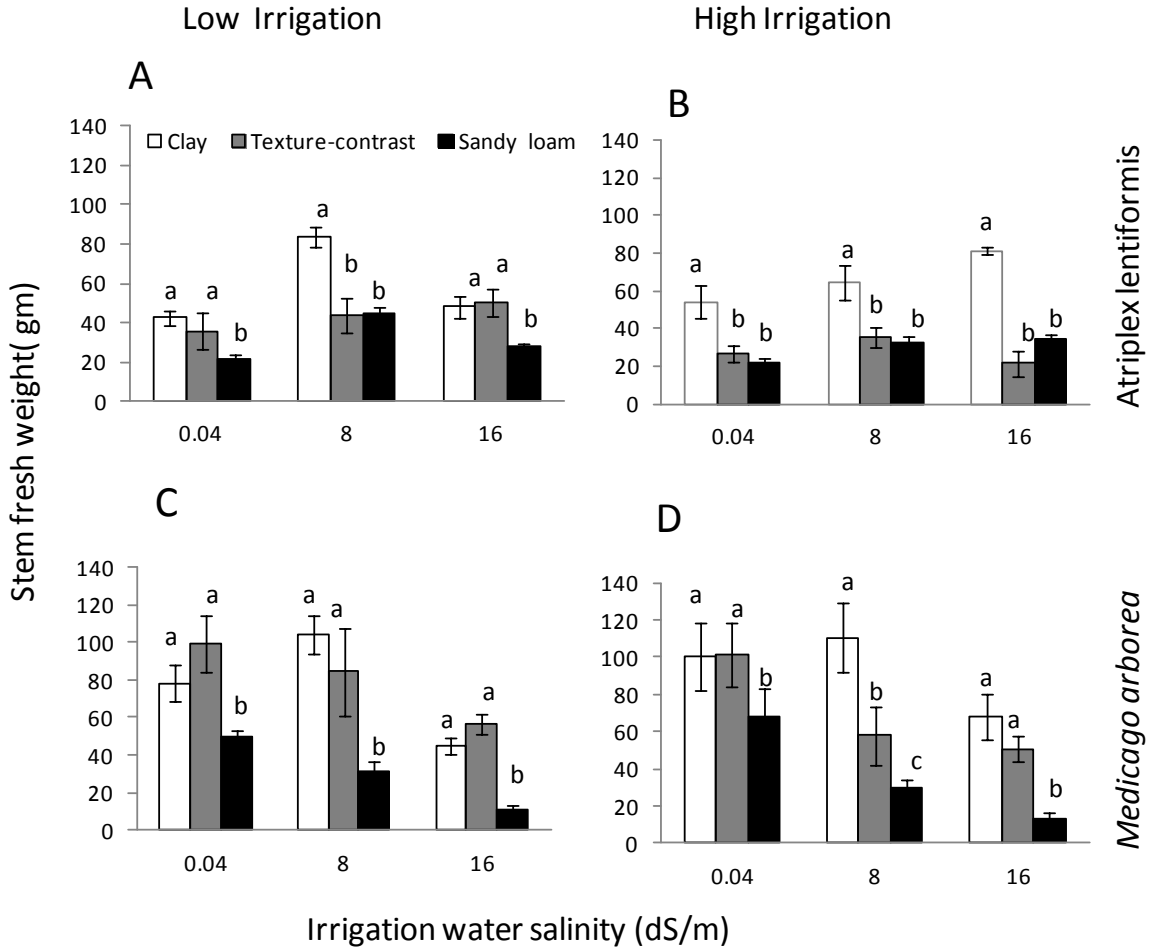

Fig. 5. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. fresh weight of stem of *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

Overall, when water salinity increased from 0.04 to 16 dS/m, the total dry biomass yield (leaf and stem) of *A. lentiformis* was increased by 1.4, 1.3 and 1.5 times in the clay, texture-contrast and sandy loam texture situations respectively but yield was significantly reduced in *M. arborea* by 1.5, 3.1 and 5 times in the same soil textures.

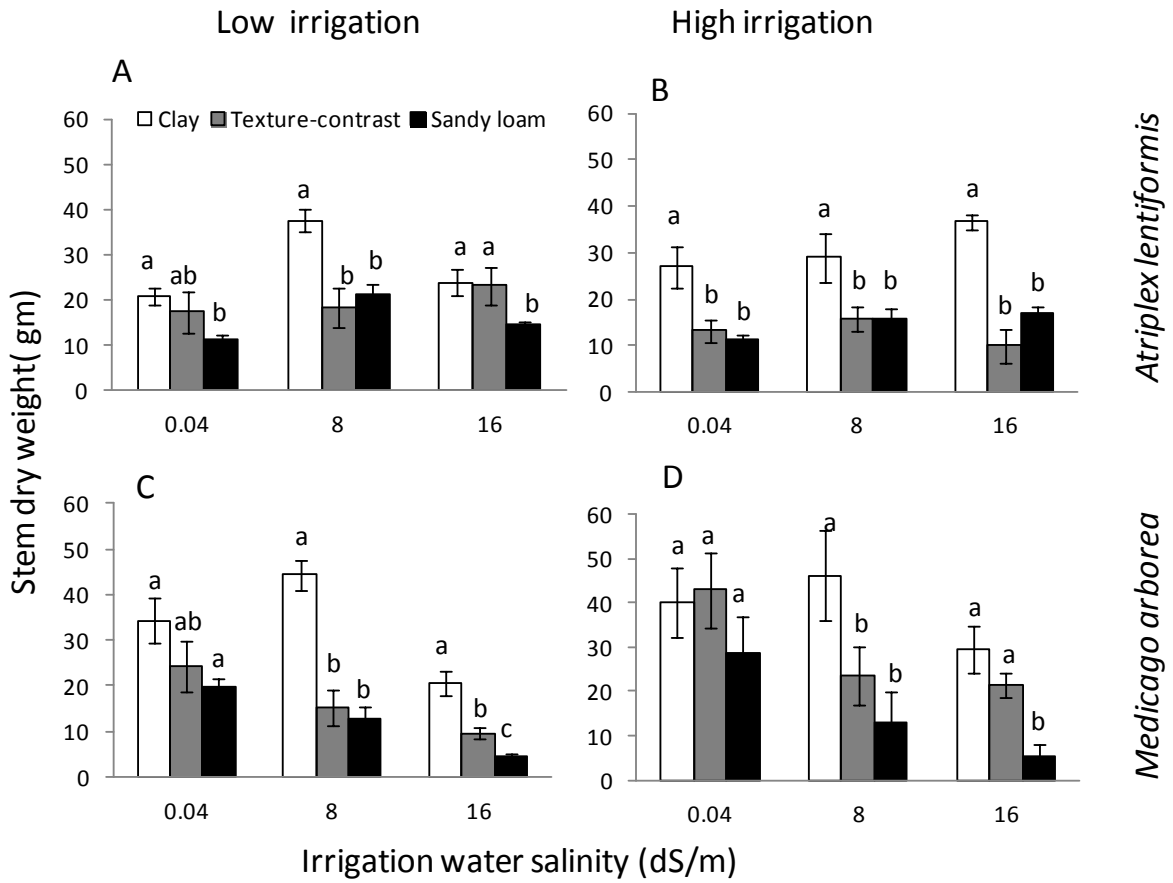

Fig. 6. Effects of irrigation water salinity (0.04, 8, 16 dS/m) on mean ± S.E. dry weight of stem of *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

3.3.3. Quantum yield of Photosystem II (PSII)

The maximum quantum yields of PSII (chlorophyll fluorescence Fv/Fm values) were above 0.72 and 0.78 in both *A. lentiformis* and *M. arborea* respectively (Fig. 7), and there was no significant difference between salinity treatments. If comparison were made based on soil textured PSII performance was always higher in the sandy loam soil for *A. lentiformis* but same trend was not observed in *M. arborea*.

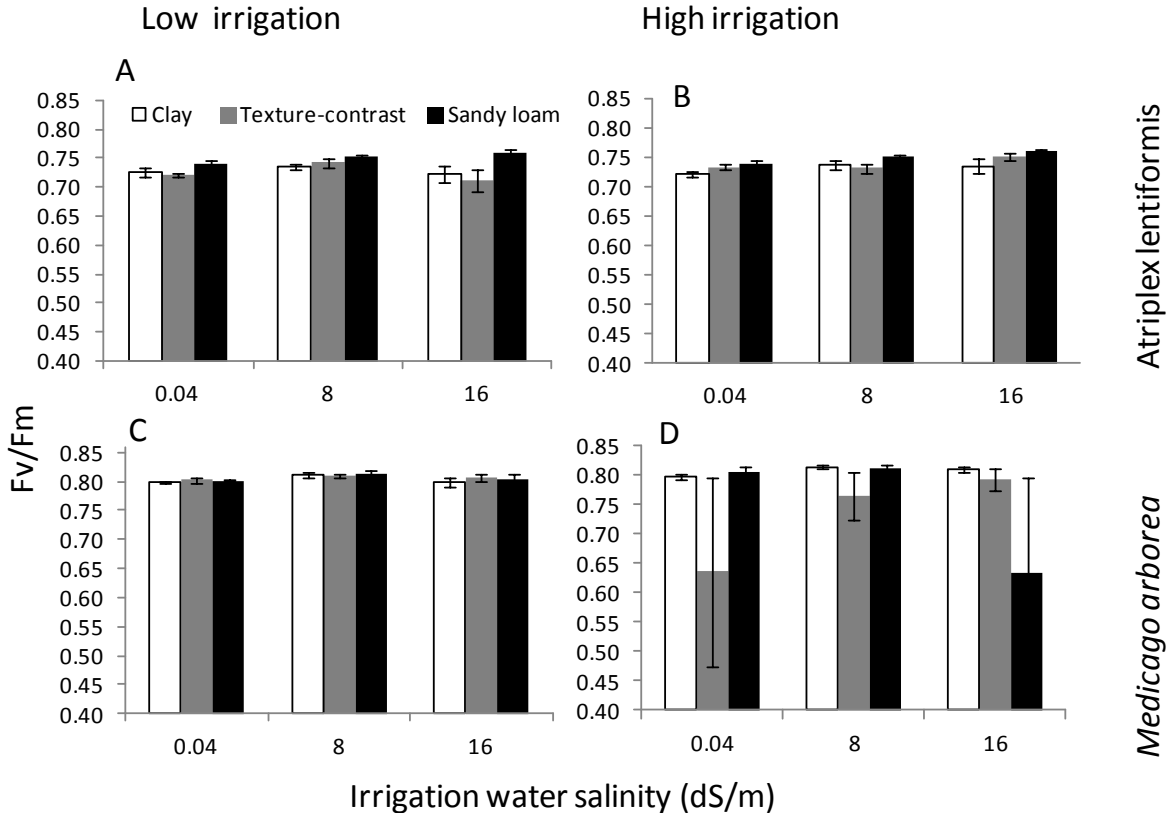

Fig.7. Changes in the maximum efficiency of PSII Photo chemistry (Fv/Fm) in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D*)*. Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

3.3.4. Stomatal conductance (gs)

There was no significant impact of salinity on stomatal conductance in *A. lentiformis* regardless the salt concentrations, soil types and irrigation levels with overall average stomatal conductance values being around 25-30 mmol $m^{-2}s^{1}$. The stomatal conductance was always lower in sandy soil compared to other soil types in the low irrigation treatment (significant at $p< 0.05$); however, it was the clay soil which had the lower values in high irrigation treatment.

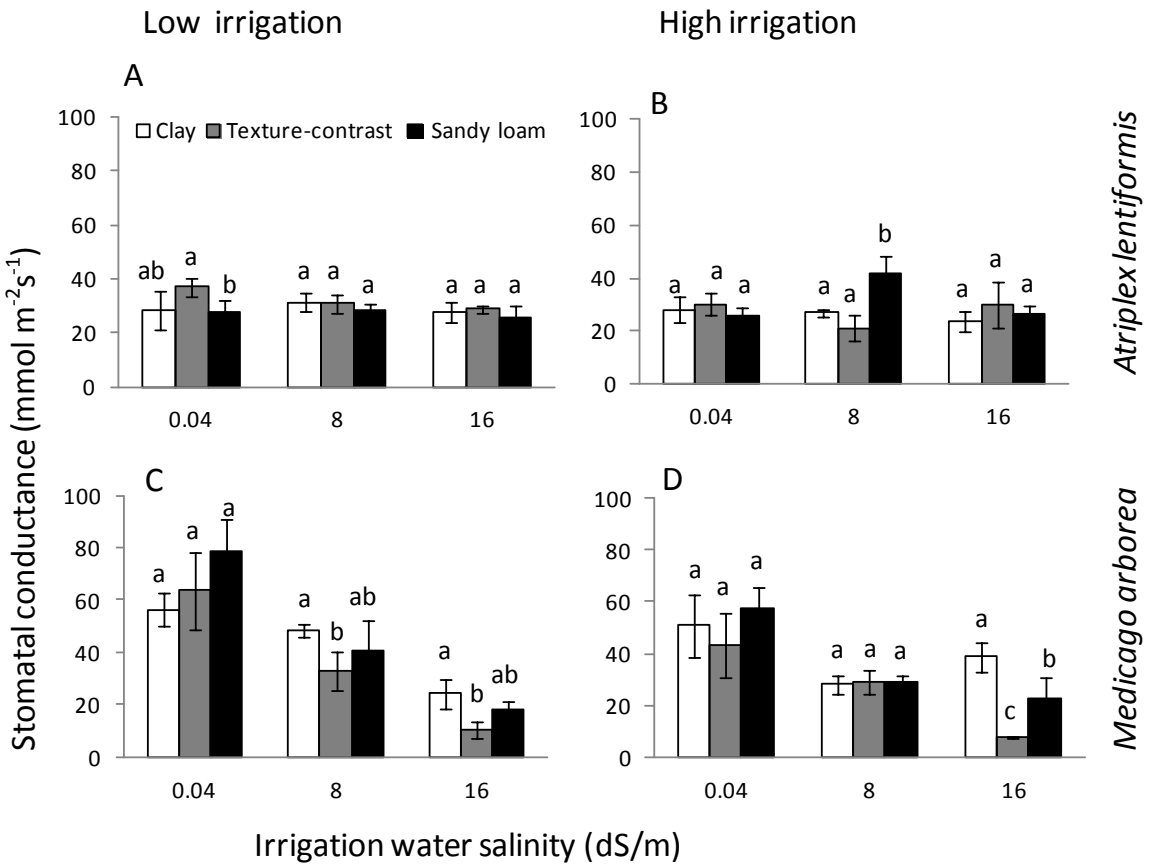

Fig. 8. Changes in stomatal conductance (gs) in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. (Low irrigation = 700 mm/year, High irrigation = 1300 mm/year).

A significant reduction in stomatal conductance at 16 dS/m was observed in *M. arborea* (Fig. 8). It is also noted that conductance was higher in the clay soil in both 8 and 16 dS/m treatments compared to other the soil texture situations. In *M. arborea*, reductions in stomatal conductance at 8 and 16 dS/m salinity levels were 41% and 74% of control respectively. The effect of irrigation levels on stomatal conductance was not significant in *Medicago arborea* ($p>0.05$).

3.3.5. Leaf chlorophyll content (SPAD measurements)

The leaf chlorophyll content is an important parameter in determining the photosynthetic rate and a sensitive indicator of plant stress. In this study, salinity had significant ($p=0.001$) impact on chlorophyll content in *A. lentiformis* but quantity of the applied water did not show such effect. It was also observed that leaf chlorophyll values were higher (non-significant) in the

sandy loam compared to the other soil textures in high irrigation in 8 and 16 dS/m treatments but the same trends were observed in clay soil in low irrigation treatments (Fig. 9).

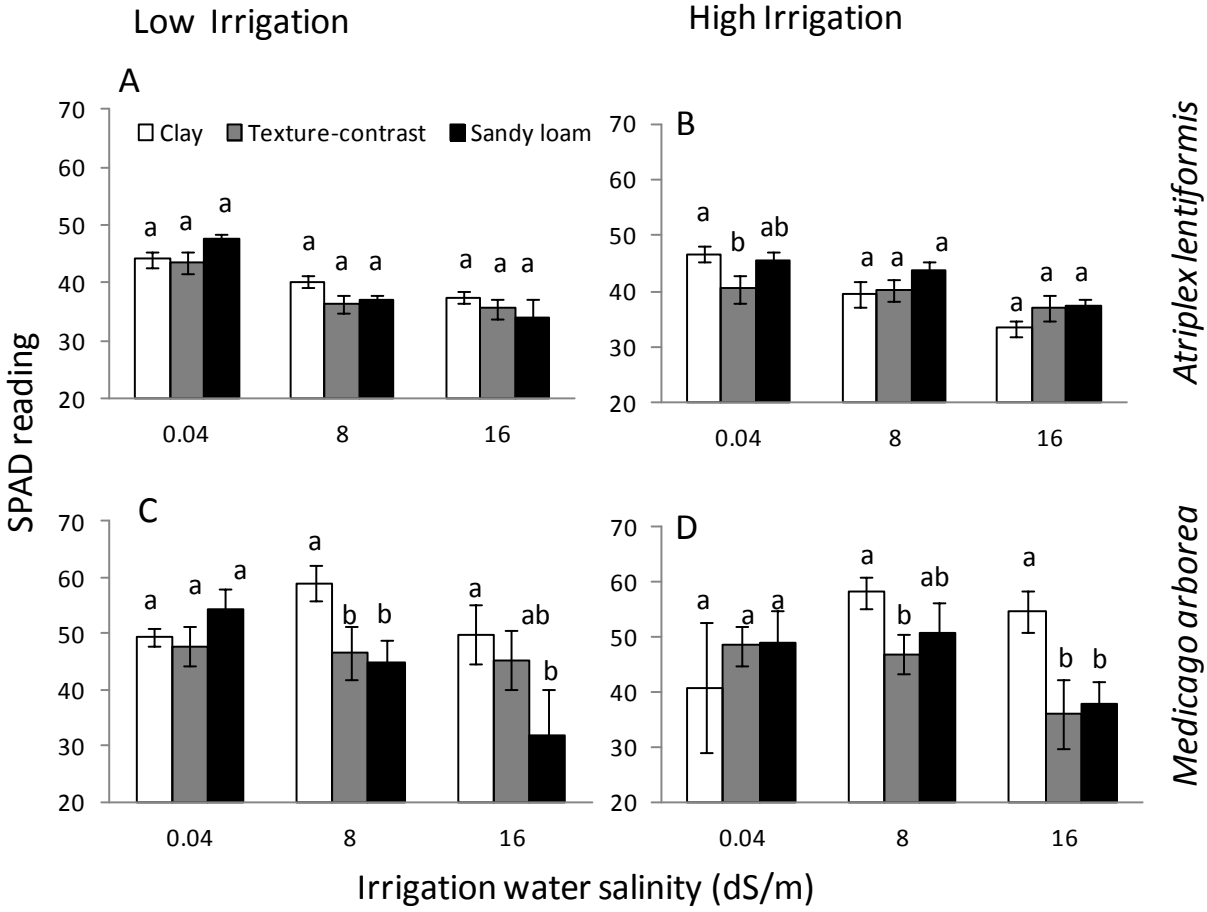

Fig. 9. Mean SPAD reading in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between soil treatments. Data is mean ± S.E. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

In *M. arborea*, leaf chlorophyll values were significantly affected by soil texture ($p = 0.0017$) and salt levels ($p = 0.0025$). The highest leaf chlorophyll value was observed in clay soil at 8 dS/m salinity in both high and low irrigations. If comparison is made within the other soil texture, the average leaf chlorophyll value was lower in sandy loam soil in both 8 and 16 dS/m water salinity. In addition, leaf chlorophyll values decreased with increasing salinity treatments in the texture-contrast and sandy soil textures in both high and low irrigation.

3.3.6. Ion concentrations

The leaf sap Na^+ increased 1.5 – 2 fold compared with control plants in *A. lentiformis*, whereas in *M. arborea* 6 and 9 fold increments were reported for plants grown at 8 and 16 dS/m salinity levels, respectively (Fig. 10). In both species, leaf Na^+ concentration was higher in the

sandy loam and lowest in the clay textured soil (significant at $p<0.05$). For *A. lentiformis* and *M. arborea* Na^+ concentration was significantly ($p<0.05$) higher with increased salinity treatment but no effect occurred between irrigation rates.

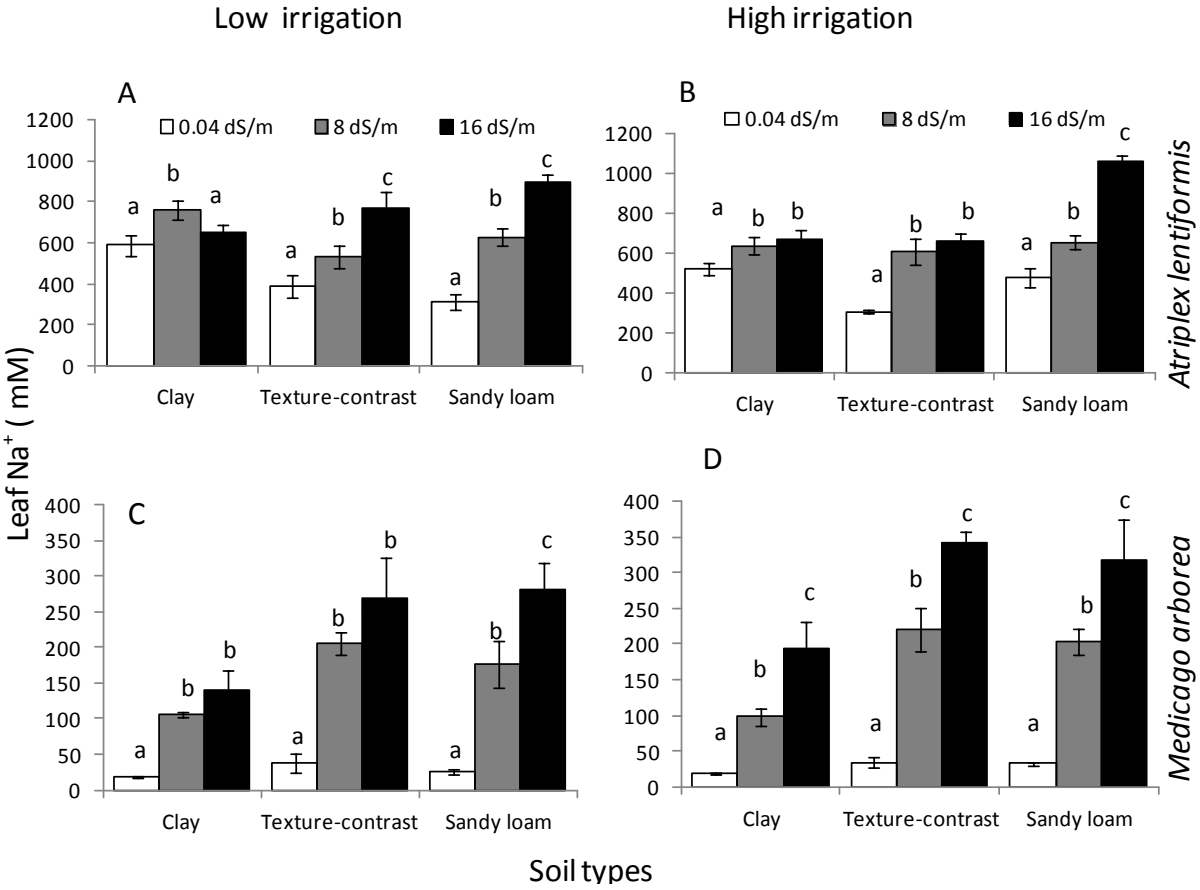

Fig. 10. Mean leaf sodium ion content in *Atriplex lentiformis* (A, B) and *Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between salinity treatments. Data is mean ± S.E. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

A decline in leaf K^+ concentration was observed when salinity levels increased in both plant species (Fig. 11). The reduction was 40 and 60% in *A lentiformis* and 50 to 60 % in *M arborea* of control at 8 and 16 dS/m salinity treatments, respectively. Plants grown in clay soil had comparatively higher K^+ compared to the sandy loam and texture-contrast soils for both species. However, the irrigation levels had no significant effect on the leaf K^+. A comparison between the two species show *A. lentiformis* had 2 – 3 fold more K^+ than *M. arborea*.

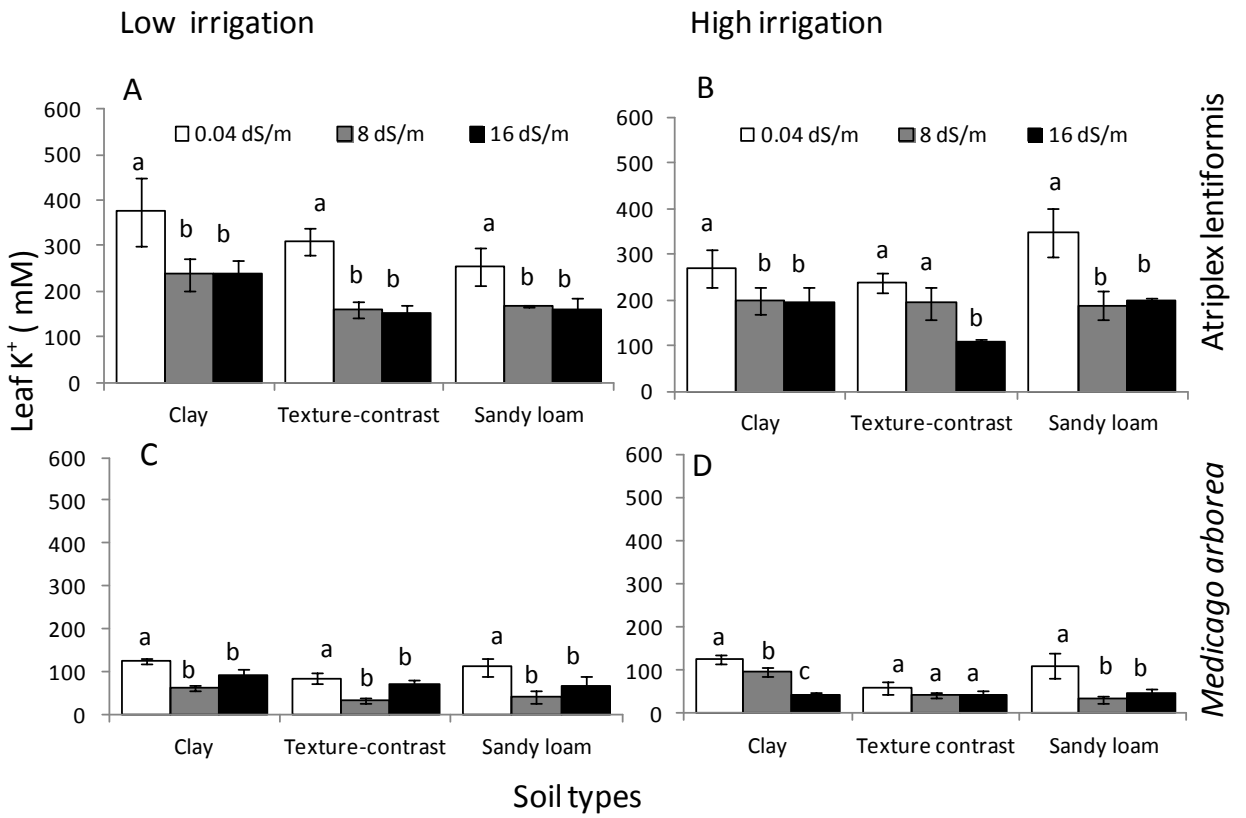

Fig. 11. Leaf K^+ content in *Atriplex lentiformis* (A,B) *and Medicago arborea* (C,D*)*. Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± S.E. (*n* = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

Leaf Cl^- accumulation was significantly increased in both species with increasing salinity treatments (Fig. 12). Average leaf Cl^- levels were 111, 439 and 636 mM in *Atriplex lentiformis* and 40, 193 and 448 mM in *Medicago arborea* at 0.04 , 8 and 16 dS/m salinity treatments, respectively (all significant at $p< 0.001$). It shows that salt-treated *A. lentiformis* had accumulated 4 and 6 times more Cl^- in its leaves compared to control, while in *M. arborea* Cl^- was 5 and 11 times more when grown at 8 and 16 dS/m salinity than in the control treatment, respectively. At 16 dS/m higher Cl^- was observed in *A. lentiformis* in the clay soil but for *M. arborea* it was higher in the texture-contrast and sandy loam textures. In general the higher Cl^- concentrations were observed in *A. lentirormis* than in *M. arborea* but the rate of irrigation had no significant effect on Cl^- accumulation in the plant leaf.

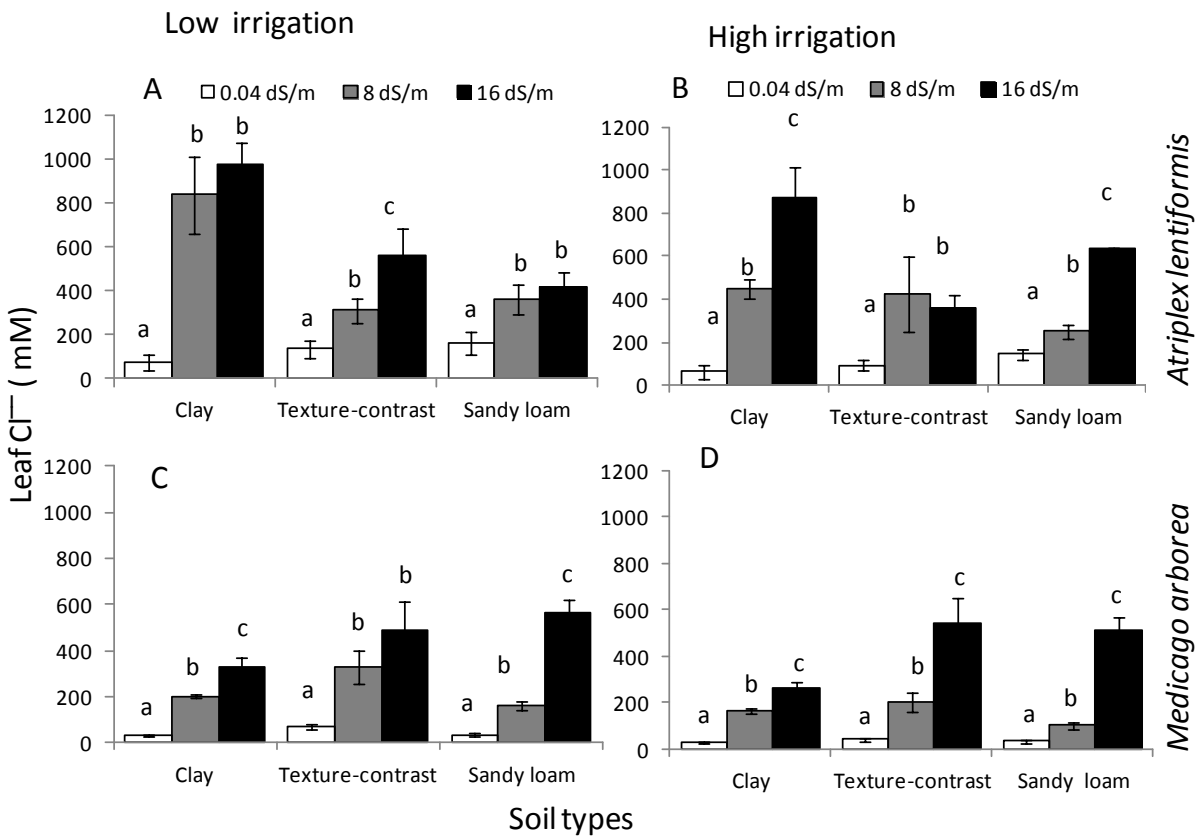

Fig. 12. Leaf Cl^- content in *Atriplex lentiformis* (A,B) and *Medicago arborea* (C, D*)*. Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± S.E. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation= 680 ml/pot/day).

3.3.7. Osmolality

Leaf sap osmolality significantly increased in salt treated plants in both species and the trend was similar in both soil types and irrigation levels (Fig 13). At the same time, a strong positive correlation ($p<0.001$) was observed between Na^+, Cl^- and osmolality (Table 2). In addition, there was a negative correlation between leaf Na^+ and chlorophyll concentration (Fig. 14) in *M. arborea* (R^2 = 0.32) and *A. lentiformis* (R^2 = 0.17). Similarly, leaf Na^+ was also negatively correlated with the stomatal conductance in *M. arborea* (R^2 = 0.41) but not in *A. lentiformis* (R^2 = 0.003).

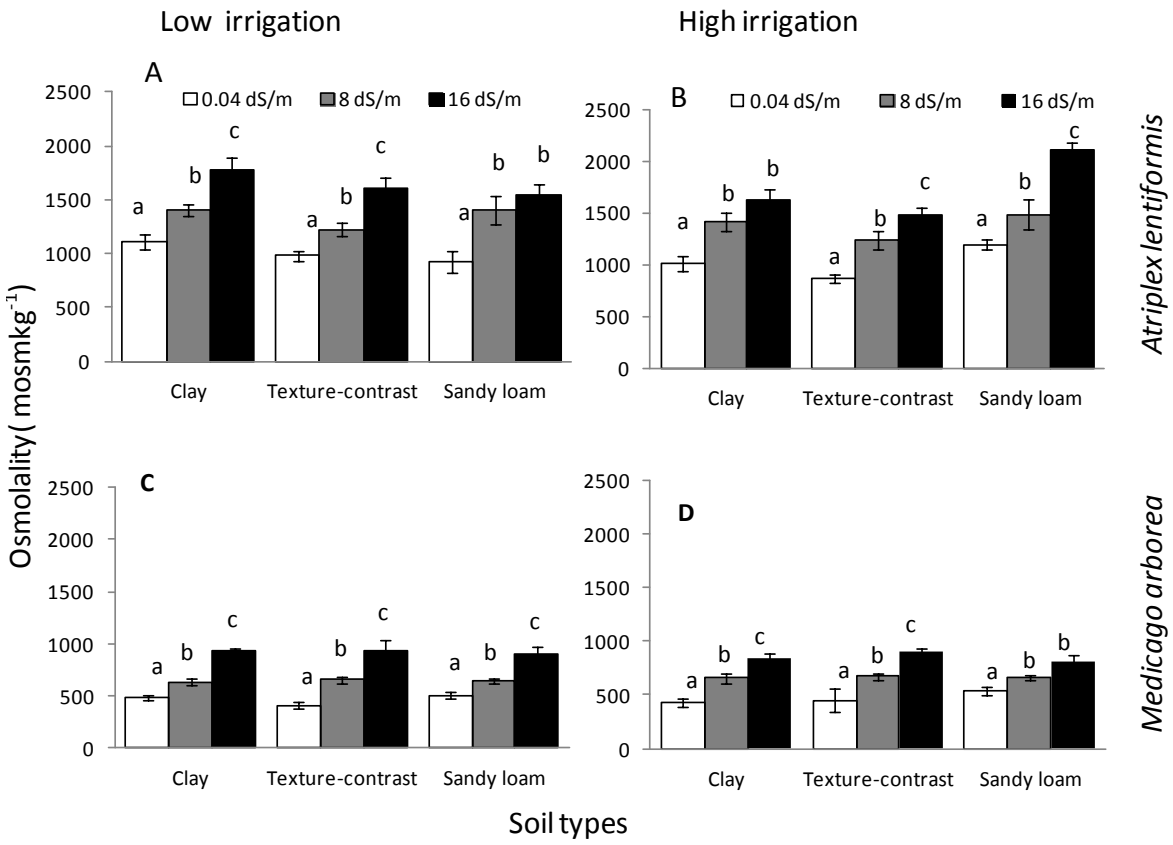

Fig. 13. Shoot sap osmolality (Osm; mmolkg^{-1}) of *Atriplex lentiformis* (A, B) *and Medicago arborea* (C, D). Different letters above bars represent significant ($p<0.05$) difference between salinity treatment. Data is mean ± S.E. (n = 5). (Low irrigation = 340 ml/pot/day, High irrigation = 680 ml/pot/day).

3.4. Discussion

3.4.1. Differential growth responses between plant species under saline condition

Atriplex lentiformis and *Medicago arborea* plants irrigated with saline water showed drastically different biomass responses, with *Atriplex* plants benefiting from the applied salt (Fig. 1) and *M. arborea* growth becoming more stunted as the level of salt increased in the irrigation water (Fig. 2). The reduction in the biomass of *M. arborea* with increased levels of salt concentration appears to be a consequence of both osmotic stress and specific ionic toxicity caused by the applied saline water. The supporting evidence for the first component comes from stomatal conductance (*gs*) data. Decreased stomatal conductance limits net photosynthesis and diversion of energy to produce compatible solutes in saline environment (Flowers et al., 2015). This conclusion is supported by the marked declining *gs* value observed in *M arborea* at high

salinity (Fig. 8) which is expected to reduce net CO_2 assimilation (Munns, 2002) and led to decreased growth rate (Fig. 2). The physiological rationale behind this reduction could be an attempt to decrease the water loss under the conditions of "physiological drought" imposed by salinity (Shabala, 2013). The closure of stomata or reduction of stomatal conductance is related to both increased ABA production under saline conditions (Ashraf and Harris, 2013; Durner, 2013) and reduced K^+ availability to maintain turgor pressure in guard cells (Anschutz et al., 2014). This effect also causes in reduction of photosynthesis with decreased diffusion of CO_2 to chloroplast (Netondo et al., 2004). Salt-treated wheat plants also show a positive relationship between stomatal conductance and relative growth rate, where higher CO_2 assimilation rate was a result of higher stomatal conductance (James et al., 2008). In this work, significantly reduced stomatal conductance in high salinity treatments in *M. arborea* indicates that plants suffered from osmotic stress. The effect was observed in reduction in biomass yield (Fig. 3 and 5). However, in *A. lentiformis* similar trend were not observed suggesting it is equipped with highly efficient mechanisms to ensure efficient control of stomata (hence, sustained CO_2 assimilation) under saline conditions.

In many salt-affected plants species the reduction of stomatal conductance at high salinity treatments results from both an inability of the plant to maintain water balance (Horie et al., 2011, Sutka et al., 2011; Shavrukov, 2013) and a reduction in the root hydraulic conductivity (Calvo- Polanco et al. 2014). The closing of stomata could be a defence mechanism of plants to avoid excessive water loss (Yang et al., 2005). Reduced stomatal conductance in salt-affected plants may also be associated with a down-regulation of PIP aquaporin gene expression (Boursiac et al., 2005). However, in *A. lentiformis* stomatal conductance was not significantly decreased, as in *M. arborea,* when plants were exposed in salinity treatments and indeed it was stable. It would therefore be interesting to examine the level of expression and the activity of aquaporins in *Atriplex* roots, and compare these with *M. arborea* plants.

Due to the similarity in physical and chemical properties between Na^+ and K^+, the former competes with the later for the binding sites thus affecting a large number of metabolic enzymes in the cytosol (Anschutz et al., 2014). This also impacts the cell elongation rate. This was not the case for *A. lentiformis* which displayed a typical halophytic response with suboptimal growth in the no salt and enhanced growth under mild to moderate salinity stress as found in previous research (Naidoo and Raghunanan, 1990; Short and Colmer, 1999). The positive correlation (R^2 = 0.41, $p<0.01$) between *A. lentiformis* leaf biomass and leaf Na^+ concentration (Table 2) suggests that Na^+ was used by *A. lentiformis* to maintain plant growth, most likely as a major

osmolyte used for turgor maintenance and cell expansion. However, this correlation was negative (R^2 = -0.49, p<0.01) for *M. arborea* indicating inability of this species to utilise Na^+.

Table 2. Correlation matrix (Pearson's r values) calculated for a range of physiological characteristic me measured in *Atriplex lentiformis* (A) and M*edicago arborea* (B) grown in different level of salinity condition.

A

	OSM	Leaf Na^+	Leaf K^+	Leaf Cl^-	LFW	SFW
OSM	n/a					
Leaf Na^+	0.80**	n/a				
Leaf K^+	-0.20	-0.16	n/a			
Leaf Cl^-	0.62**	0.54**	-0.18	n/a		
LFW	0.44**	0.41**	-0.28*	0.65**	n/a	
SFW	0.18	0.21	-0.04	0.45	0.81	n/a

B

	OSM	Leaf Na^+	Leaf K^+	Leaf Cl^-	LFW	SFW
OSM	n/a					
Leaf Na^+	0.80**	n/a				
Leaf K^+	-0.22	-0.55**	n/a			
Leaf Cl^-	0.70**	0.76**	-0.32**	n/a		
LFW	-0.45**	-0.49**	0.04	-0.44**	n/a	
SFW	-0.53**	-0.52**	0.08	-0.45**	0.88	n/a

*Correlation is significant at 0.05 level; ** Correlation is significants at 0.01 level. OSM, Osmolality of leaf sap; leaf Na; Leaf Na concentration; Leaf K , Leaf K concentration, leaf Cl, leaf Cl- concentratin; LFW, leaf fresh weight; SFW, Stem Fresh weight.

Another possible aspect worth discussing is the presence of highly dense and multilayered epidermal bladder cells in *Atriplex* species (Shabala, 2013). Traditionally, the role of these bladder cells has been attributed to external sequestration of excess $Na^{+,}$ taking it away from the metabolically important mesophyll cells. As the diameter of epidermal bladder cells is often approx. 10-fold bigger than epidermal cell, they could sequester 1000-fold more Na^+ compared with epidermal cells (Shabala and Mackay, 2011). However, these bladders may have been playing an important role as a secondary epidermis to reduce water loss when exposed to saline environment (Adams et al., 1998). In addition to this, some reports suggest that halophyte species may substitute K^+ for Na^+ to increase turgor pressure rapidly in stomatal guard cell (Shabala and Mackay, 2011) which can be justified by the higher concentration of K^+ in *A. lentiformis* compared to the *M arborea* (Fig. 11).

Plant growth can also be adversely affected by both high cytosolic Na^+ or Cl^- concentrations and low cytoplasmic K^+ (Yeo, 1998; Chen et al., 2008; Smethurst et al., 2008; Demidchik et al., 2010; Shabala, 2009; Flowers et al., 2015). The low K^+ concentration at 8 and 16 dS/m salinity in both plants indicates that either there was a restriction of the K^+ uptake, or poor K^+ retention in plant tissues (Fig. 11). The K^+ retention ability of plants has been linked to the salinity tolerance in some plant species (Chen et al., 2008; Smethurst et al., 2008). A reduced cytosolic K^+ concentration can negatively affect cell metabolism (Shabala and Cuin, 2008) and cause programmed cell death due to the activity of caspase-like proteolytic and endonucleolytic enzymes in the salt affected plants (Demidchik et al., 2010, Shabala, 2009). However, the reduction of K^+ concentration in *A. lentiformis* was not below the optimum level of cytosolic K^+ (100 – 150mM) needed for protein synthesis in plant (Walker et al., 1998). On the other hand, reduction of K^+ concentration in *M. arborea* was below this threshold. So this may be one of the reasons for reduction of *M. arborea* growth in saline treatments while such impact was not noticeable in *A. lentiformis* under same conditions.

The chlorophyll fluorescence (Fv/Fm) data provided further insights into the physiological responses of these plants to salinity treatments. Our results reveal that there was no significant reduction in Fv/Fm values in both plant species when grown in up to 16 dS/m salinity (approximately 160 mM). Similar to the earlier suggestions (Lu and Zhang 1998; Lu et al., 2003; Redondo-Gomez et al., 2006; Shabala et al., 2013), this result suggests that there was no damaged to PSII photochemistry due to applied levels of salinity and that the PSII system is not a main target of salinity stress. However, in more sensitive species such as faba bean 10 dS/m NaCl treatment has resulted in a significant decline in Fv/Fm values (Tavakkoli et al., 2010). A similar decline was also observed in *M. arborea* at higher irrigation salinities (200-300 mM; Boughalleb et al., 2009a,b). Thus, there is some apparent threshold in the extent PSII can be protected against the detrimental effects of salinity.

The shoot biomass may also be influenced by the level of chlorophyll pigments in the plant leaf, as the amount of sequestered carbon (hence, yield) is generally proportional to the overall pigment content in the shoot (Ramesh et al., 2002; Islam et al., 2014). This statement can be supported by our observation where leaves with high chlorophyll content had high biomass yield in *M. arborea* (Fig. 3) as high chlorophyll content resulted in high photosynthesis rate. However, effects of salinity of pigment composition and chlorophyll content are not straightforward. While elevated cytosolic Na^+ and Cl^- may result in both reduce rate of chlorophyll biosynthesis and its faster degradation (Tavakkoli et al., 2010; Tavakkoli et al., 2011; Flowers et al., 2015), salinity also results in the smaller cell size and hence more dense pigment

"packing". So, ultimately it is the total shoot chlorophyll content and the number of functional PSII units that matters for leaf photochemistry.

3.4.2. Impacts of soil texture on plant growth

Our results show that *A. lentiformis* and *M. arborea* grown in clay textured soil had higher biomass compared with the other soil texture situations regardless of the salinity or irrigation treatments. One reason for this higher growth could be the higher nutrient status measured in the clay soil used compared to the pure sandy loam or the sandy loam over the clay. What this study also shows is the low vs high irrigation rates had less impact than either the texture or salinity levels. This suggests waterlogging was not a significant issue. In *M. arborea*, which is a moderately salt-tolerant plant, a decline in biomass was expected in the high salinity situation but not in the same pattern in all soil texture set-ups. The physical properties of the soils, both soil texture and structure, may have played a role in the plant's performance because these soil properties affect the soil's nutrient holding capacity, aeration, pore space water-holding capacity and drainage (Warrence et al., 2002; Brady and Weil, 2009). Sandy soils generally have high infiltration rates and lower nutrient and water-holding capacity (Brady and Weil, 2009) whereas clay soils can hold more water but generally have slower infiltration and drainage rates. We suggest that in the sandy texture the applied water drained out more rapidly than the clay or the sandy loam/clay situation. Thus sandier soils would generally be preferable for high saline water irrigation (Warrence et al., 2002). However the high volumes of water and their salt content applied in this study may have leached more key plant nutrients out of the sandy loam than the clay textured media. This was shown in our field trials under high salinity irrigation (Chapter 6). This might explain the lower biomass yields of both species observed in the sandy loam despite this soil being more "immune" to salinity built up.

In general, in both species, clay-soil-grown plants had higher chlorophyll content and stomatal conductance compared to either the sandy loam over clay or the sandy loam soil in low irrigation (Fig. 8 and 9). We argued that soil structure and nutrient holding capacity may have been playing a role in this effect. Plants may have benefitted by the naturally high nutrient content of the clay soil (Table 1). In addition to this, it was also noted that in 16 dS/m saline treatment, lower leaf Na^+ concentration was obtained from clay soil compared to other soil types in both plant species. It could be possible that some of the Na^+ in the salt applied was absorbed in the soil particles and became less available for the plant for take up (e.g. shifting the ratio between exchangeable and solution Na^+). It has been reported that soil texture is strongly

correlated with a soils ability to adsorb or desorb chemical ions (Miller and Donahue, 1995). Clays provide cation exchange sites in soils and consequently are also a source for excess exchangeable Na^+ leading to swelling and dispersion (Miller and Donahue, 1995). It is also well known that Na^+ will accumulate in clay soils as opposed to sandy soils because of their lower leaching fraction and higher soil surface area (Warrence et al., 2002). It was also observed that in high salinity conditions *M. arborea* grown on clay soil had less leaf Cl^- concentration than plants grown on other soil types but just the opposite result was found in *A. lentirofmis*. Clay soils are predominantly negatively charged and applied Cl^- (via NaCl) is likely to be repelled (Bohn et al., 1979) and become easily available or leached through the soil. In general, halophytes maintain turgor by accumulating high Cl^- concentrations of 340 to 475 mM in plant tissues. Conversely, in glycophytes it is regarded as minor component of their cell sap osmotic pressure and has concentration of 7 to 70 mM (White and Broadley, 2001). This could be one of the reasons for having higher Cl^- in *A. lentiformis* compared to *M. arborea* that were grown in clay soil at the same salinity levels.

3.4.3. Tissue tolerance maybe conferring salinity tolerance in *A. lentiformis* but not in *M. arborea*

In this study, the overall plant performance of *Atriplex lentiformis* was better than the *Medicago arborea* up to the 16 dS/m water salinity despite the fact that overall Na^+ concentration was 2 – 3 times lower in *M. arborea* than *A. lentiformis*. This suggests an efficient tissue tolerance mechanisms were operating in *A. lentiformis*. Higher Na^+ and Cl^- values in A. *lentiformis* can be described as a typical characteristic of halophytic plants where they were known to accumulate Na^+ and Cl^- concentrations >500 mM (Flowers, 1985) to maintain a positive turgor under saline conditions. Some halophytes such *Tecticornia* species may accumulate close to 2M Na^+ on a tissue water basis when grown under highly saline conditions (English and Colmer, 2013). Shoot tissue tolerance in halophytes may be conferred by several different mechanisms (Shabala and Mackay 2011; Shabala, 2013; Shabala et al., 2014; Flowers et al., 2015) and these include: efficient vacuolar sequestration in leaf mesophyll; Na^+ sequestration in photosynthetically inactive tissues (such as parenchyma cells in succulent plants); Na^+ sequestration in external structures such as epidermal bladder cells (EBC); and Na^+ excretion through salt glands or glandular trichomes. The role of each of these mechanisms may differ depending on the species and its habitat. As *Atriplex* species contain well-developed dense layer of EBC on both surfaces of the leaf (Shabala and Mackay 2011; LoPresti, 2014; Shabala et al., 2014), it is plausible to suggest that external Na^+ sequestration may be the major contributor

to the tissue tolerance in this species. It was showed earlier for a related *Chenopodium quinoa* species (Bonales et al., 2013 a, b) that salt tolerance in young quinoa leaves possessing dense salt bladders was conferred predominantly by preferential Na^+ loading in EBC, while in old leaves where EBC were less numerous and not functional vacuolar Na^+ sequestration in leaf mesophyll was playing the critical role. In both cases, plants were efficient at removing the excessive Na^+ away from metabolically active cells of the growing plant body and minimised the effect of salinity (Shabala et al., 2014). We believe a similar scenario may be applicable for Atriplex species as well.

In the present study high level of Na^+ and K^+ ions in *A. lentiformis* observed in control plants (0.04 dS/m) indicates that these ions were used to maintain osmotic adjustment and probably plants accumulated those ions from the existing soil without supplemented by irrigation waters. Consistent with this notion, A. *lentifornis* had much higher Na^+ concentrations at low irrigation salinities compared with *M. arborea* but did not increase uptake much when concentration of salt was increased in irrigation water. This was not the case for *M. arborea*. Hence, control of the xylem Na^+ loading and its transport to the shoot has also contributed to differential growth patterns of species under saline conditions. The kinetics of this process warrants a separate investigation.

The ability of halophytes to sequester Na^+ in vacuoles is essential to adjust osmotic pressure and maintain positive shoot turgor and enable growth of new tissue (Glen et al., 1999; Flowers and Colmer, 2008). At the molecular level, the ability of halophytic plants to sequester huge amount of Na^+ in their vacuoles is related to the expression of tonoplast Na^+/H^+ antiporters under saline conditions (Barkla et al., 1995; Glenn et al., 1999, Apse and Blumwald, 2007). Importantly however, the activity of this exchanger has to be energized by either tonoplast H^+-ATPases (Vera-Estrella et al., 1999; Wang et al., 2001) or H^{+-} PP-ases (Krebs et al., 2010). Shabala (2013) suggested that overexpressing Na^+/H^+ NHX exchangers can only be fully functional under saline conditions if plants do not heavily invest available ATP pool for the production of compatible solutes (which otherwise used for fuelling tonoplast H^+-ATPase) and possess efficient K^+ retention mechanism (to enable tonoplast H^+-PPases to function). These assumptions seem to be met by *A. lentiformis* but not by *M arborea*. Indeed, *A. lentiformis* had a high tissue K^+ concentration compared to *M arborea* in salinity treatments and was, therefore, more capable of maintaining (K^+-dependent; Rea and Poole, 1993; Shabala 2013) operation of the tonoplast H^+-PPase. On the other hand, *M. arborea* grown at 16 dS/m salinity had higher Na^+ accumulation rate and low K^+ concentration. Based on the above assumption, it can be suggested that due to lack of sufficient K^+ (and, hence a failure to energize the tonoplast H^+-PPases) *M.*

arborea may not have an ability to compartmentalise Na^+ within tissues by intracellular storage. So these accumulated high concentrations of Na^+ (in the metabolically active areas in the leaves) may have damaged the photosynthesis apparatus in *M. arborea.* As a result photosynthesis process reduced and growth is stunted.

Chapter 4

Temporal changes in soil properties and plant characteristics of *Atriplex* species and *Medicago arborea* when grown in different soil types under saline irrigation*

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Abstract

Salinity stress tolerance is a complex polygenic trait composed of numerous sub-traits which allow plants to adapt to adverse environments. While each of them is important for the overall plant performance, they all operate at very different timescales. Thus, the timing aspect of salt stress development and adaptation should be always kept in mind. The aspect of timing should be also taken into account when describing physiological changes in the root rhizosphere. The aim of this work was to compare changes in the soil profile and physiological characteristics between *Atriplex lentiformis* (halophyte) and *Medicago arborea* (glycophyte) species exposed to prolonged NaCl treatments and grown in various soil types (as described in a previous chapter). The main research question was: do the processes of adaptation to salinity differ between the two species, and which physiological mechanism explains a better long-term overall performance of *Atriplex* species? Plant (leaf sap Na^+, K^+ and Cl^- concentration, leaf sap osmolality, chlorophyll content, stomatal conductance) and soil (pH, soil and leachate electrical conductivity) characteristics were measured at regular (monthly) intervals for up to five months of salinity treatments and then correlated with each other. Our major conclusions are as follows: (1) The overall poor performance of *Medicago arborea* plants grown under saline conditions was associated with the following factors: (i) its strong reliance on organic osmolytes (hence, associated carbon costs) for osmotic adjustment; (ii) poor K^+ retention that compromised stomata opening; (iii) its inability to prevent xylem Na^+ loading; and (iv) its poor shoot tissue

tolerance, most likely due to inability to provide efficient Na^+ sequestration in vacuoles. (2) While soil salinity (ECse) was significantly increased with increasing water salinity (ECw), ECse of a sandy loam soil was ~50 % less of ECw in the high irrigation rate suggesting a possibility of the long-term usage of saline irrigation in soils with low clay content.

Key words: salinity; Atriplex; Medicago; plant performance; kinetics; saline irrigation; Soil texture, osmolytes.

4.1. Introduction

Salinity stress tolerance is a complex polygenic trait composed of numerous sub-traits which allow plants to adapt to adverse environments. These include control of Na^+ uptake and sequestration within the root and its delivery to and accumulation in the shoot (Hasegawa et al., 2000; Tester and Davenport, 2003; Munns and Tester, 2008). Salinity stress tolerance is also ultimately dependent on the plant's ability to osmotically adjust to altered water availability in the soil (Munns 2002; Munns and Tester, 2008) as well as the ability of the plant cells to maintain cytosolic K^+ homeostasis (Shabala and Pottosin 2014; Anschutz et al., 2014) and their ability to prevent formation of detoxify/scavenge reactive oxygen species (ROS) accumulated in plant tissues under saline conditions (Miller et al., 2010; Bose et al., 2014a,b). Each of these mechanisms operates on its own timescale, ranging from milliseconds (e.g. salt stress sensing; Shabala et al., 2015) to weeks and months (ion toxicity in the shoot; Munns 2002). As each of them may be important to overall plant performance, the timing aspect of salt stress development and adaptation should be always kept in mind.

The most obvious symptoms of the salt stress are reduced plant growth. The physiological mechanisms behind this reduction may differ, however, depending on the time after stress onset (Munns 2002). The decrease in the leaf elongation rate in the first 10 days of salinity treatment was virtually the same for salt sensitive durum wheat and salt tolerant barley (Munns et al., 1995) suggesting that the primary reason for this reduction was osmotic effect of salinity stress. Indeed, a very rapid (within minutes) reduction of leaves expansion rate was observed in rice (Yeo et al., 1991), maize (Neumann, 1993), wheat and barley (Passioura and Munns, 2000). After a day of salinity treatment, leaf and root growth rate reached a reduced steady rate once the specific Na^+ toxicity effects start to take place (Munns, 2002). The timing of this event varies dramatically between species (most likely as a result of their ability to control

Na^+ delivery to the shoot and also on their vacuolar Na^+ sequestration capacity). In the salt sensitive species the effect of salinity becomes noticeable within a week, with green leaves turning into yellow and excised from plant. If the old leaves die at a higher rate than the new leaves are produced then there is a struggle for the plant to survive. In some salt-sensitive species such as white lupin salt stress-specific effects became visible after a few days of salinity treatment (Munns, 1988). In salt tolerant plants and genotypes, toxicity symptoms become evident only after several weeks (Wu et al., 2015). Salt toxicity can be seen first in the older leaves compared to young ones as more Na^+ and Cl^- will be delivered to transpiring leaves via transpiration stream. However, as salt stress passes from weeks to months, a progressive leaf death will continue and new leaf formation process continues in perennials but in annuals it becomes more about survival and plants switch on the reproductive system and initiate flower production while there is still some ATP left. For example, in cereals salinity causes less floret per ear and alters the flowering and maturity time (Munns and Rawson, 1999).

However, the above view is somewhat oversimplified and fails to take into account specificity of salt stress sensing and adaptation at the cellular level. For example, specific Na^+ toxicity in the shoot is usually attributed to detrimental effects of reactive oxygen species (ROS) accumulated in shoot tissues because of a number of stimuli including salinity stress (Apel and Hirt 2004 ; Kwak et al., 2006) and this leads to progressive oxidative damage and ultimately cell death (Sharma et al., 2012). Under saline conditions ROS production occurs in both leaf and shoot (Mittler, 2002; Miller et al., 2008) very rapidly (within minutes) and causes significant effects on cell metabolism. Arabidopsis plants exposed to salinity of 100mM NaCl resulted in 2.5 to 3-fold whereas at 250 mM it was 4 to 4.5 fold increased in hydroxyl radical generation (Demidchick et al., 2014). The impact of ROS includes direct activation of Ca^{2+} permeable plasma- membrane cation influx channels in plant cells triggering rapid Ca^{2+} uptake (Pei et al., 2000; Demichik et al., 2003). Similarly, activation of certain class of K^+ permeable NSCC channels are also thought to be associated with ROS (Demichik et al., 2003), which causes massive K^+ leak from the cytosol and depletion of cytosolic K^+ concentration (Shabala et al., 2006). Then, the continuous elevation of Ca^{2+} and depletion of cytosolic K^+ stimulate caspase-like protease and trigger programmed cell death (PCD) (Shabala, 2009). Importantly the PCD was observed within an hour after imposing the stress in plant roots. Thus, kinetics of stress development should be always discussed in a strict tissue-specific context.

Even faster are the mechanisms behind salt stress signalling. Each major environmental constraint that plants experience under saline conditions e.g. elevated Na^+ levels in the rhizosphere, reduced water availability due to the hyperosmotic stress, a dramatic increase in

reactive oxygen species (ROS) accumulation in plant tissues and a massive disturbance to the cytosolic Ca^{2+} and K^+ homeostasis are all sensed by either membrane-bound or cytosolic sensors and then translated into a broad array of physiological and genetic alterations that optimise plant performance under saline conditions (Shabala et al., 2015). As plant membranes contain numbers of transport proteins which may act as salt sensors but these sensor proteins operate on different time scales. The fastest are mechano-sensing systems which can activate within millisecond upon alteration in solute osmotic potential. After that tentative proteins, such as SOS1, and voltage-gated H^+ -ATPase/GORK tandem systems which operate in a few seconds are then followed by slower sensing systems which rely on changes in concentrations of second messengers (such as cyclic nucleotides, or extracellular ATP) found in cytosol or apoplast. All of these sensing mechanisms may be integrated by cytosolic free Ca^{2+} and H_2O_2 and these components enable plants to decode information about the specific nature and severity of the stress and provide it into stress specific Ca^{2+} and H_2O_2 "signatures" (Shabala et al., 2015).

Surprisingly, very little is known about specificity and time-dependency of salt stress sensing in signalling between halophytes and glycophytes. Although both halophytes and glycophytes utilises Ca^{2+} sensors (such as CaM, CMLs, CDPKs, and CBL/CIPKs) but they are regulated in different way especially the kinetics of the Ca^{2+} (Shabala et al., 2015) and H_2O_2 signalling (Bose et al., 2014a,b). NaCl-induced cytoplasmic Ca^{2+} elevation was more rapid in halophytic quince (*Cydonia oblonga* Mill) (D'Onofrio and Lindberg, 2009) than glycophytic rice (Kader et al., 2007). Also, only in rice Na^+ entry into the cytoplasm was required for Ca^{2+} elevation (Kader et al., 2007) suggesting that in a halophytic quince Na^+ was sensed at the *apoplastic* side (Shabala et al., 2015). Similarly, in the halophyte *Cakile maritime* leaves salt-induced H_2O_2 build up was peaked after 4 h of imposing salinity treatment but declined sharply afterwards, whereas in *Arabidopsis thaliana (*glycophyte), H_2O_2 accumulation continued even beyond 72 h of salt application (Ellouzi et al., 2001).

The importance of the time factor to understand the mechanisms conferring plant adaptation to salinity may be also illustrated by comparing responses to salt between pea (salt-sensitive) and barley (salt-tolerant) plants. Despite being classified as salt-sensitive species, pea plants showed no symptoms of the salt stress and performed even better than barley after one week of salt stress (Bose et al., 2014b). This superior performance and ability of tolerating a high level of NaCl in the short term was attributed to pea's pronounced ability to maintain highly negative membrane potential (much more efficient than barley). However, as this process is energy demanding and was also not accounting for detoxification of accumulated ROS species, this strategy has failed in the longer term, and after 4 weeks of 160 mM NaCl treatment pea

plants were effectively killed, while in barley plants only some moderate symptoms of salinity stress (light chlorosis and reduction in growth rate) were observed (Bose et al., 2014b). What about halophytes? Which strategy do they use? Do they behave like "stayers" and economise the energy resources, or do they fight salinity from the very beginning?

The aspect of timing should be also taken into account when describing physiological changes in the root rhizosphere. Indeed, if SOS1-mediated Na^+ exclusion from uptake is a preferred strategy to fight salinity (as shown for many plants – e.g. *Arabidopsis*, Zhu et al., 1998; Apse et al., 1999; Moller et al., 2009; wheat - Byrt et al., 2007; James et al., 2011; rice - Ren et al., 2005) then one should expect a gradual build-up of salinity in the root zone. This will increase downhill Na^+ concentration gradient and put more and more pressure on roots to extrude Na^+ from cytosol. So, how long can plants keep doing it? Is there any difference between the kinetics of ion accumulation (or depletion – as for essential nutrients) in the root zone between halophytes and glycophytes? Are there any of these processes dependent on the soil type?

The aim of this work was to fill some of the above gaps in our knowledge and compare changes in the soil properties and physiological characteristics between *Atriplex lentiformis* (halophyte) and *Medicago arborea* (glycophyte) species exposed to prolonged NaCl treatments and grown in various soil types (as described in a previous chapter). The main research question was: do the processes of adaptation to salinity differ between two species, and which physiological mechanism explains a better long-term overall performance of *Atriplex* species?

4.2. Materials and methods

The plant materials and seedling growing conditions, experimental set up and design, and methods of non-destructive physiological measurements (e.g. stomatal conductance, SPAD, and chlorophyll fluorescence), osmolality and tissue ion content (Na^+, K^+ and Cl^-) were described in previous chapter 3. The chemical analysis of the soil was conducted using air dry soil that passed through 2 mm sieve. The soil electrical conductivity (EC) and pH were measured using 1:5 soil/distilled water mix solution stirred for 1 hour at 25°C according to Rayment and Higginson (1992). The measured EC values were then converted into EC values for the saturated paste extract as described elsewhere (Sonmez et al., 2008). After initiation of the salinity treatments leachate was collected at monthly interval by placing a saucer under the pots shortly before the next irrigation application. Leachate was then collected and stored at 4^0C in the air tight glass containers until analysis. EC and pH were measured on unfiltered samples by using

handheld EC and pH meter (Model WP-81 Cond, TDS, Sal, pH, ORP, Temp. meter, TPS Pty Ltd, Brisbane Australia).

4.2.1. Data analysis

The statistical significance of difference between means was determined by the Student's t-test and the significance of correlations between different parameters was determined by bivariate correlations based on Pearson correlation (two-tailed). Regardless of treatments data were pooled together from each measurement time and correlated between selected parameters by using excel 2007 data analysis tool.

4.3. Results

4.3.1. Soil salinity

The first question we have asked was of whether the long-term (5 months) salinity treatments result in a progressive salinity build-up, given the limited pot size? If yes, how much is it, and was this process depended on the soil type (texture)? As expected, soil salinity (measured as the electrical conductivity of the saturated soil extract, ECse,) was significantly ($p<0.01$) increased with increasing levels of salinity in irrigation water. ECse values were higher in clay and texture-contrast (the clay fraction) soils compared to a sandy loam soil. At 16 dS/m water salinity treatment, the clay soil salinity did not exceed the salinity level of irrigation water when irrigated at high irrigation rate (680 ml/pot/day) and ECse of the same soil was nearly 1.5 times higher in the low irrigation level (340 ml/pot/day) (Fig. 1). Moreover, at the highest irrigation and salinity rate (16 dS; 680 ml/day) soil ECse in the sandy loam soil was only 5.5 ± 0.3 dS/m, e.g. 3-fold lower than salinity of irrigation water (Fig. 1). ECse of the texture-contrast soil showed that salt was leached through the sandy layer and trapped in the clay soil. It was also observed that the accumulation rate was higher in low irrigation rate compared to high irrigation rate. Lower irrigation rates resulted in higher salinity build-up in the soil. For example, at the highest salinity rate (16 dS/m) the clay EC_{se} values were 13 dS/m for the high irrigation rate, and 2-fold higher (26 dS/m) for the lower irrigation rate, despite half the amount of salt being added to each pot in the latter case.

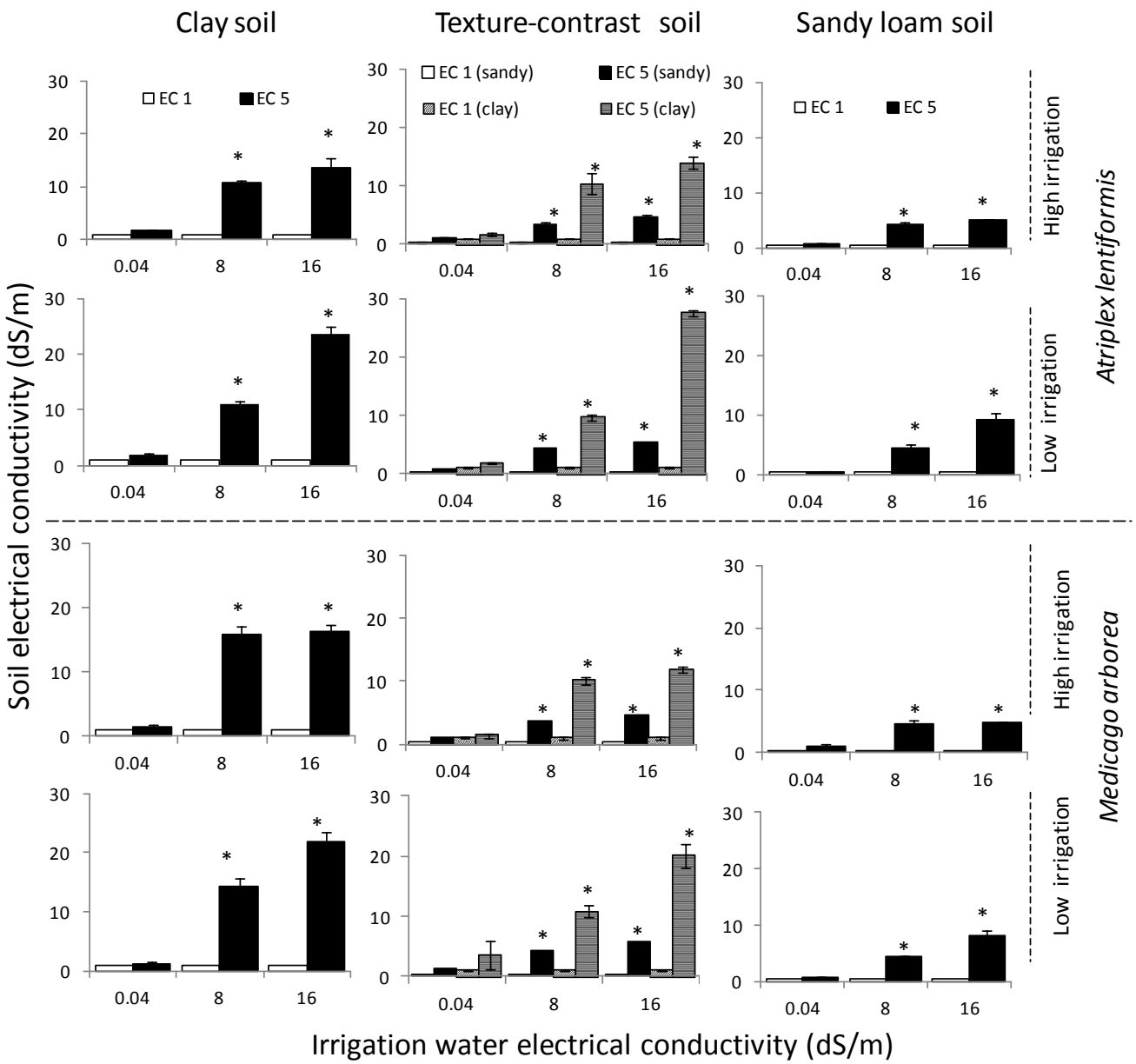

Fig. 1. Changes in the soil electric conductivity (ECse) after 5 months of saline water irrigation in different soil types. EC 1 and EC 5 indicate saturated paste extract conductivity values of the soil before and after five months after saline irrigation treatment. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml/pot/day, respectively. * indicates a significant difference ($p<0.01$) between soil EC 1 and soil EC 5 within each salinity treatment.

4.3.2. Leachate salinity

Leachate EC values were rather stable with time, except for a few case of inconsistent changes. Fig. 2 indicates the trends of EC with respect of time for the leachate samples collected from all soil and water treatments. Under control conditions (0.04 dS/m treatment) when no salt was added to the irrigation water, the EC of the leachate was almost constant with time. At 16 dS/m water salinity treatment, leachate EC of the clay soil containing *Atriplex lentiformmis* plants increased from 25 ± 1 dS/m (in first month) to 30 ± 1.5 dS/m (by the end of experiment) at low irrigation; no such trend was observed at the high irrigation rate in the same soil (Fig. 2). In the sandy loam soil the leachate EC was significantly ($p<0.05$) increased over time for both irrigation rates. Such trend, however, was not apparent in the leachate from *Medicago arborea* plants for the same treatments. Similarly, in the texture-contrast soil, leachate EC from *Medicago* plants decreased from 30 ± 1.5 dS/m to 25 ±1.5 dS/m over the duration of experiment at low irrigation, whereas in *Atriplex lentiformis* grown pots this trend was opposite.

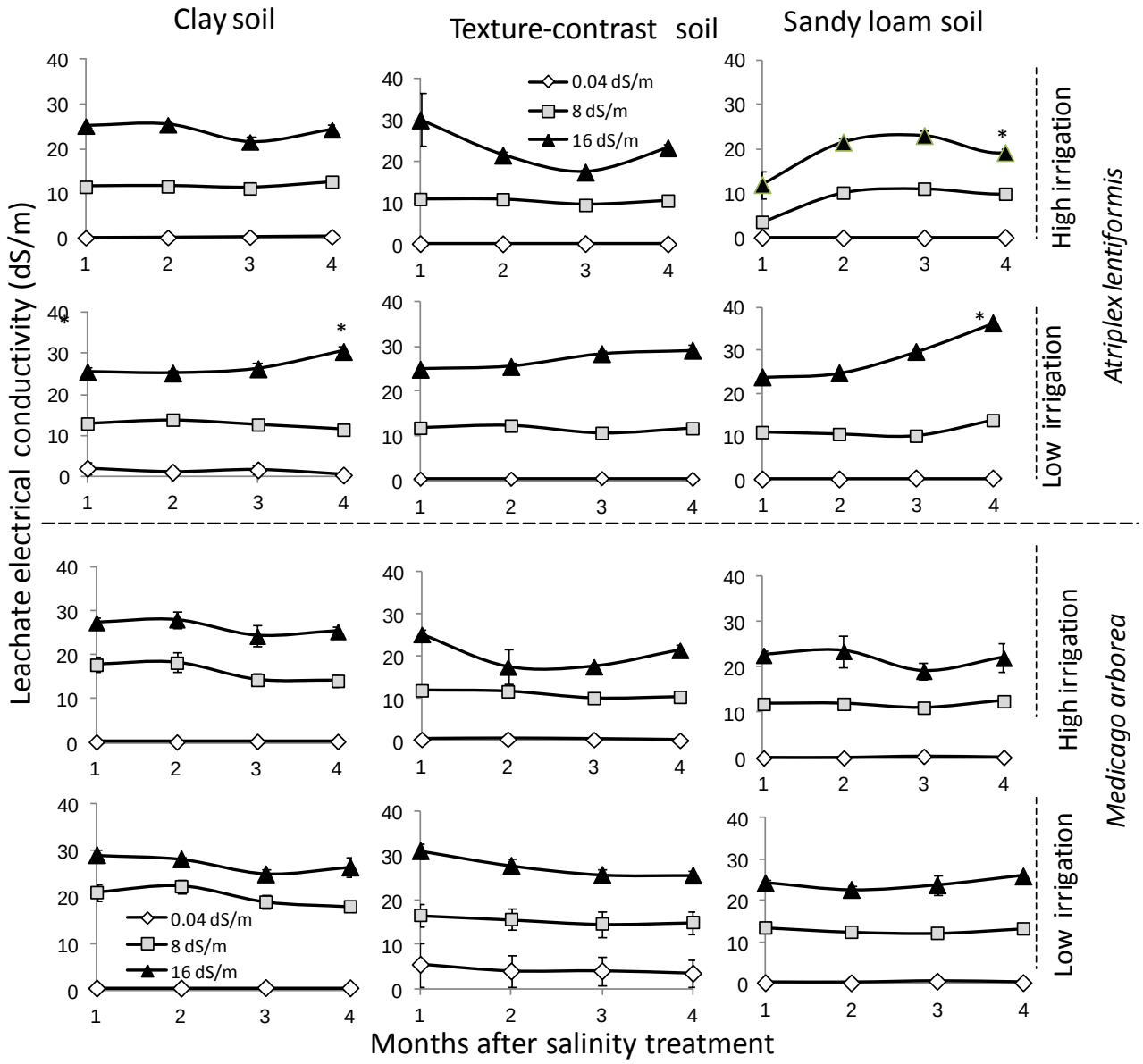

Fig. 2. Changes in the leachate electrical conductivity (EC) over the duration of experiment. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml /pot/day, respectively. * indicates significant difference ($p<0.05$) between leachate EC 1 and leachate EC 4 within salinity treatment.

4.3.3. Changes in soil pH

The soil pH has slightly increased with increasing salinity levels in the irrigated water (Fig. 3). This increase was largely independent of the irrigation rate. For example, for *Atriplex lentiformis* plants grown in the clay soil, soil pH increased from 6.5 ± 0.03 to 7.1 ± 0.07 with increasing salinity at the high irrigation rate (680 mL/pot/day), and from 6.5 ± 0.03 to 6.8 ± 0.09 at low irrigation rate (340 mL/pot/day). In all treatments, soil pH values were significantly ($p<0.05$) higher at 16 dS/m, compared to 0.04 dS/m for the same soil (Fig. 3). Soil pH increased

with time in saline water irrigated soils but this was not consistent in all soils and irrigation rates. After five months of saline water irrigation, regardless of the irrigation levels and species grown, pH values ranged from 6.4 to 7.1 in the clay soil and 5.1 – 6.0 in the sandy loam. The soil pH value in the texture-contrast soil decreased in the control (0.04 dS/m) but not in the salinity treatments.

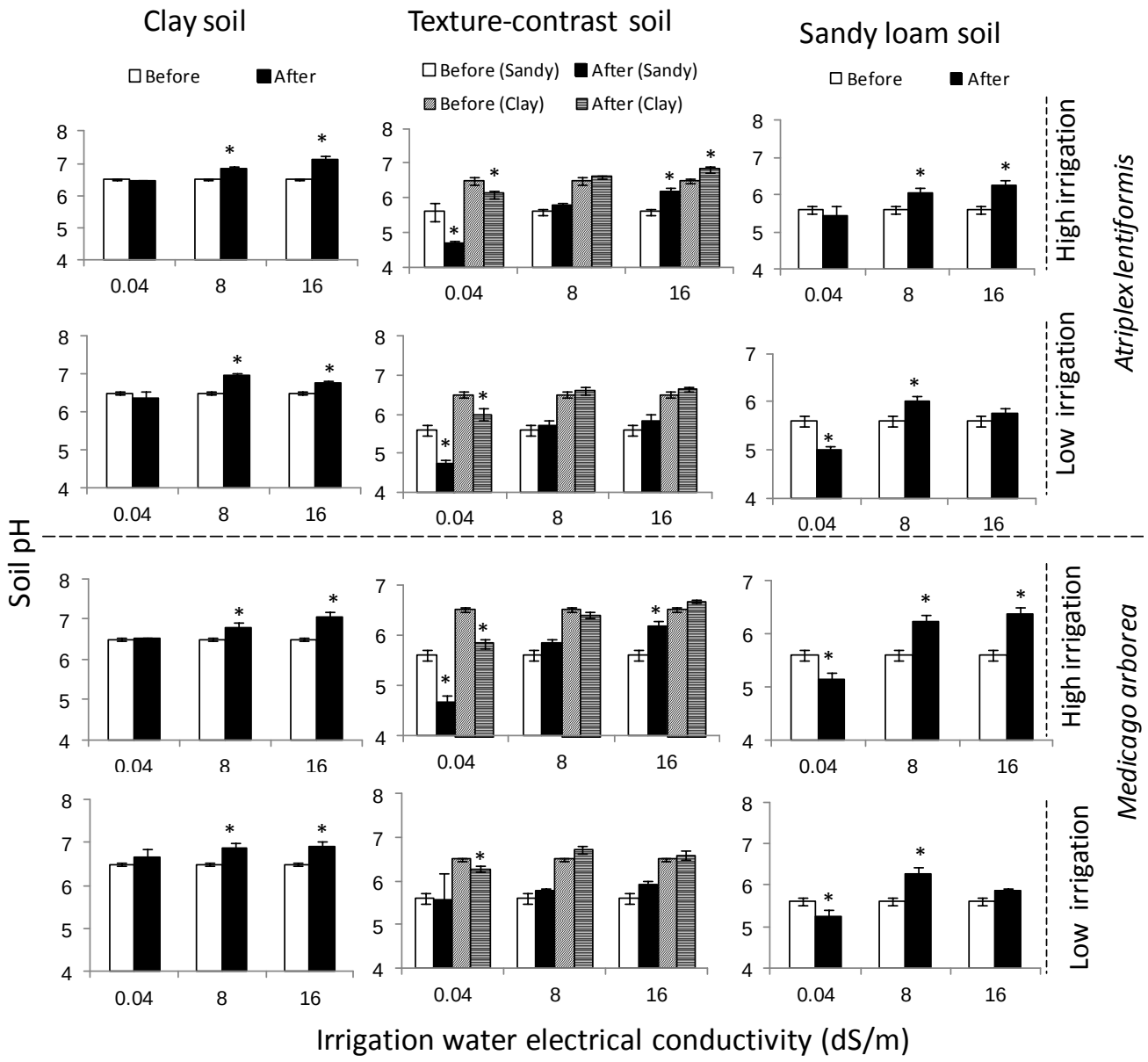

Fig. 3. Changes in the soil pH value of different soils in salinity treatments (0.04, 8.0 and 16 dS/m). Values are mean ± S.E. (n = 5). Before = before treatments; and After = after 5 months of salinity treatments, depth 0 – 15 cm = sandy loam topsoil, depth 15 – 30 cm = clay soil, low irrigation and high irrigation means water was applied at 340 and 680 ml/pot/day, respectively. * indicates significant difference ($p<0.05$) between before and after pH measurements within each salinity treatment.

4.3.4. Na^+ concentration in leaf

The kinetics of xylem Na^+ loading was investigated by assessing the amount of Na^+ delivered to the leaves by the transpirational stream over 5 months duration by collecting leaf sap at monthly intervals after initiation of salinity treatments (Fig. 4). As expected, in both plants species, leaf Na^+ content was increased with the increasing salinity of the irrigation water. This increase, however, was very different between two species. No statistically significant (at $p<0.05$) difference was found in the leaf Na^+ content between *Atriplex lentiformis* plants grown at 8 dS/m and 16 dS/m treatments, regardless of the soil type and irrigation rate, with reported values around 600 mM (Fig. 4). In the *Medicago arborea* plant, this difference was high i.e., 2-fold (e.g. 114.1 ± 23.2 vs 235.9 ± 23.6 for high irrigation clay-grown plants after 5 months of treatment). In *Medicago arborea* there was a continuous Na^+ accumulation and significantly ($p>0.05$) increased over time (Fig. 4). As a result, after five months of 16 dS/m salinity treatment, leaf Na^+ concentration in clay-grown *Medicago arborea* plants was 1.8 fold and 2.5 fold higher than in the first month measurement at high irrigation and low irrigation, respectively (Table 1). In the sandy loam soil this increase was 1.5 and 2 fold higher in the same treatments, respectively (Table 1). In the texture-contrast soil, 1.7 and 2.2 fold difference was reported for the same irrigation treatment condition (Fig. 4; Table 1). However, this progressive accumulation of leaf Na^+ was not observed in *Atriplex lentiformis* plants (Fig. 4; Table 1). Here, leaf Na^+ was not significantly different between the first and fifth month measurement in either clay or texture-contrast soils under both high and low irrigation rates (Fig. 4; Table 1). Moreover, in the sandy loam soil, leaf Na^+ concentration even decreased with time (Fig. 4). In comparison, *Atriplex lentiformis* plants grown in the sandy loam soil accumulated twice the amount of Na^+ than *Medicago arborea* plants (Fig. 4).

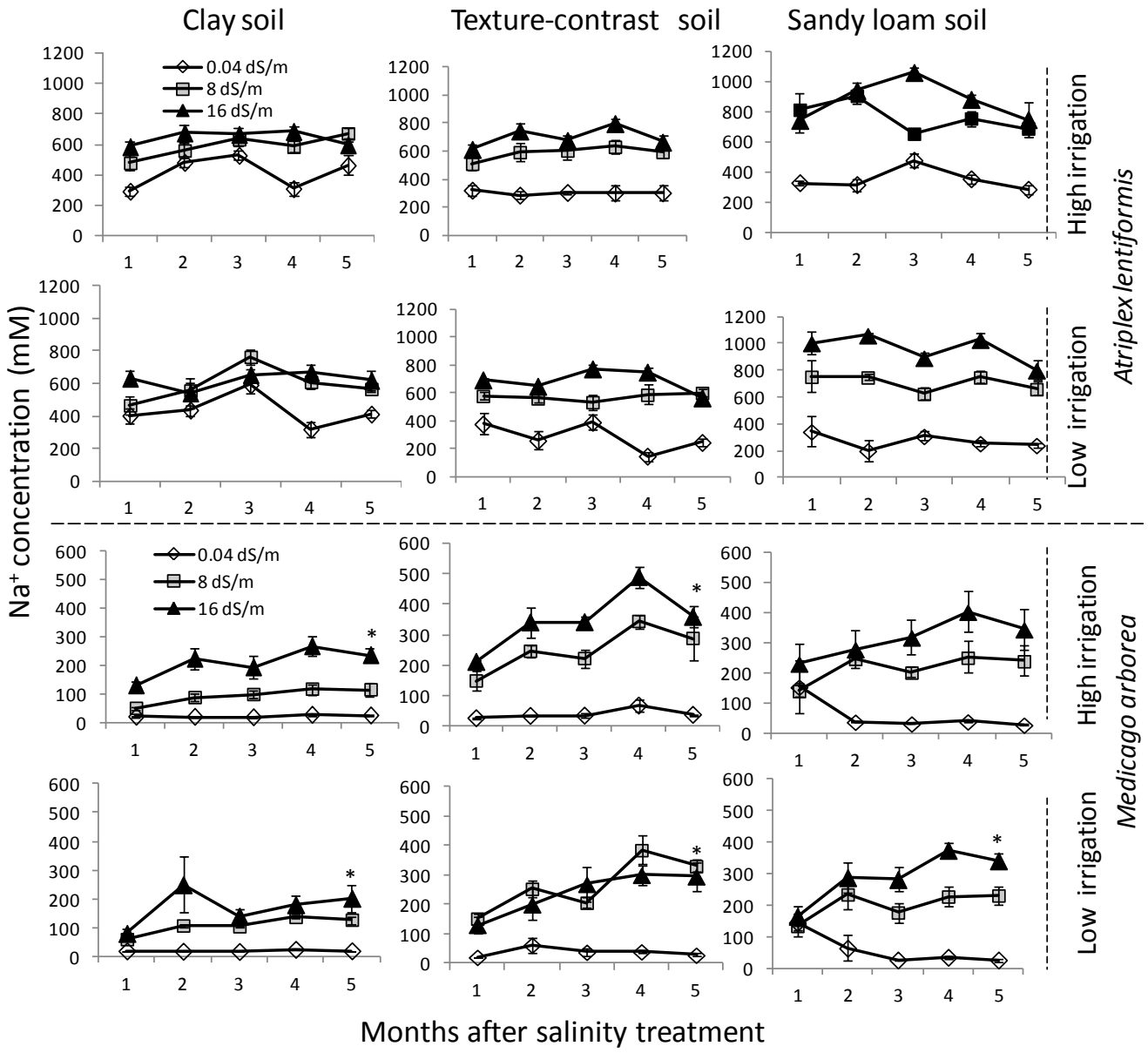

Fig. 4. Changes in the leaf sap Na^+ concentration of *Atriplex lentiformis* and *Medicago arborea* over a period of time. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml/pot/day, respectively. * indicates significant difference ($p<0.05$) in the leaf Na^+ concentration between the first and fifth measurement within salinity treatment.

Table 1. Changes in the leaf Na^+ and K^+ concentration between first and fifth measurement for 16 dS/m salinity treatment.

Irrigation level	Soil type	Leaf Na^+ after 1 month of treatment, Na^+(1)(mM)	Leaf Na^+ after 5 month of treatment, Na^+(5)(mM)	Na^+ (5) as % Na^+ (1)	Leaf K^+ after 1 month of treatment, K^+(1)(mM)	Leaf K^+ after 5 month of treatment, K^+(5)(mM)	K^+(5) as % K^+(1)
			Atriplex lentiformis				
High	C	588.0	600.3	102.1	181.6	135.3	74.5
	TC	629.6	456.2	72.5	208.8	55.7	26.7
	SL	741.8	801.5	108.1	238.5	104.3	43.7
Low	C	631.8	618.7	97.9	225.2	161.0	71.5
	TC	694.4	565.2	81.4	211.9	99.7	47.0
	SL	1004.5	801.5	79.8	364.0	83.1	22.8
			Medicago arborea				
High	C	130.5	235.9	180.7	118.5	26.2	22.1
	TC	210.5	358.0	170.1	97.9	52.5	53.6
	SL	231.5	344.8	149.0	112.1	46.4	41.4
Low	C	81.3	202.9	249.6	181.7	119.7	65.9
	TC	129.4	294.1	227.3	113.2	55.0	48.5
	SL	175.1	339.8	194.1	124.3	57.1	46.0

Notes: C, Clay; TC, Texture- contrast and SL, Sandy loam

4.3.5. K^+ concentration in the leaf

Atriplex lentiformis plants were able to retain more K^+ in their leaves when exposed to salinity, compared with *Medicago arborea* (Fig. 5). A five-fold difference in the net K^+ accumulation in leaves was observed between clay soil grown *Medicago* and *Atriplex* plants after 5 months of exposure to 16 dS/m NaCl water treatment (*Medicago*; 26 ± 4 mM and *Atriplex*; 135 ± 27 mM, significant at $p<0.05$). At the last measurement, leaf K^+ concentration of salt treated *Atriplex* plants was higher in the clay soil compared to other the soil texture set-ups (soil types). Notably, kinetics of NaCl induced K^+loss in both species was significant ($p<0.05$) with time. At 16 dS/m salinity, leaf K^+ declined by 3 fold (300 ± 15 vs 93 ± 8 mM; significant at $p<0.05$) in the sandy loam but the reduction was only 1.4 fold (203 ± 12 vs 136 ± 8 mM) in the clay soil between the first and last measurements. Similarly, in *Medicago* the K^+ reduction for the same treatment was 3.2 (118 ±10 vs 36 ± 4 mM) and 5 fold (157 ± 12 vs 30 ± 5 mM) in sandy loam and clay, respectively (Fig. 5; Table 1). K^+ concentration in *Medicago* leaves was as low as 30 – 40 mM in 16 dS/m irrigation water salinity. In addition, leaf K^+ loss was slightly higher (although not significant) in the high irrigation rate compared to low irrigation rate (Fig. 5). In statistical terms, after five months of salinity treatment the clay soil grown *Atriplex lenformis* plants retained more K^+ in the leaves compared to sandy loam soil grown plants (74.5 % vs 43% of first measurement) but it was 22% and 41.5% for the same treatment in *Medicago arborea* (Table 1).

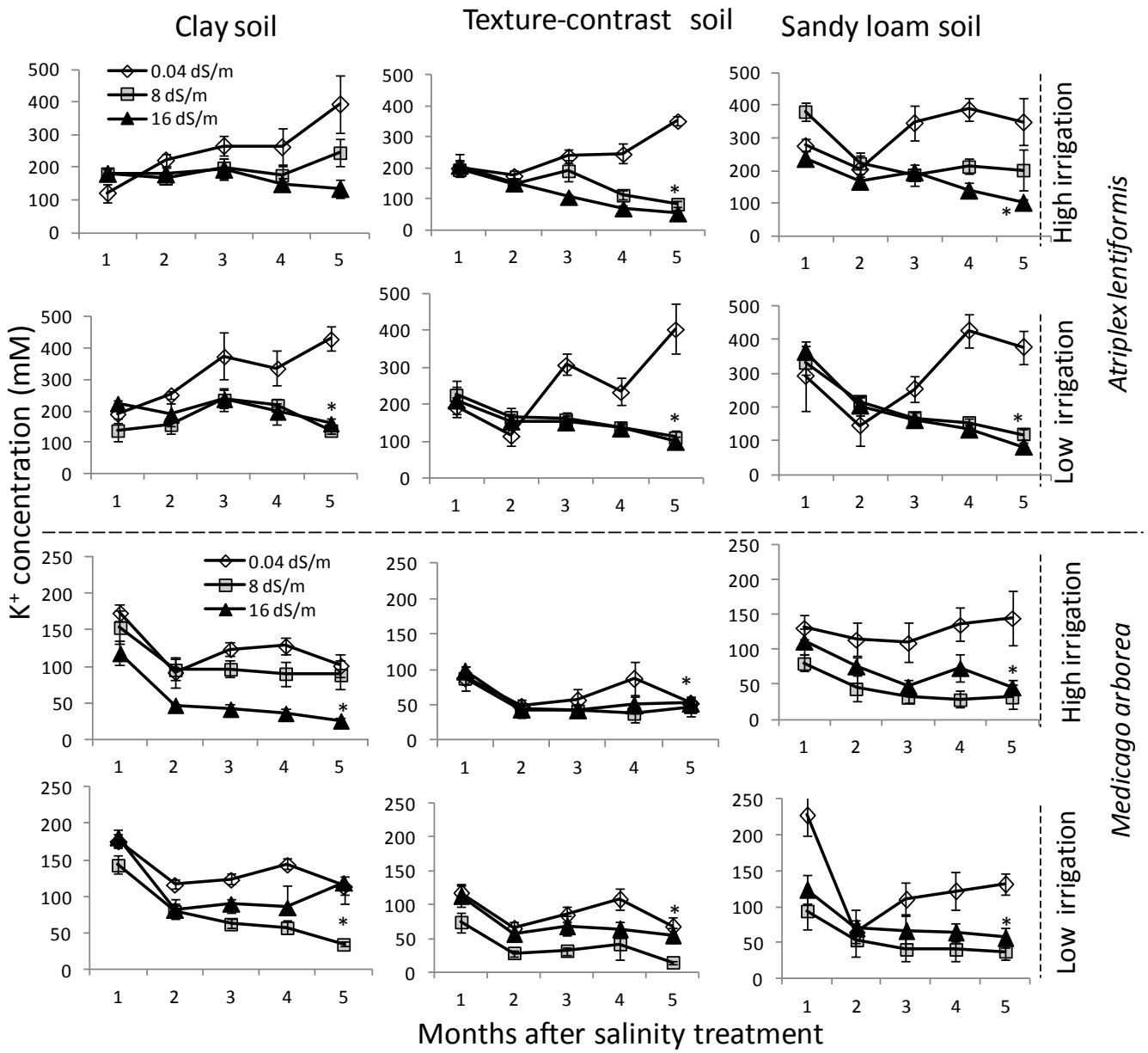

Fig. 5. Changes in the leaf sap K^+ concentration of *Atriplex lentiformis* and *Medicago arborea* over a period of time. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml/pot/day, respectively. * indicates significant difference ($p<0.05$) in leaf K^+ concentration between first and fifth measurement within the salinity treatment.

4.3.6. Changes in SPAD reading (chlorophyll measurement)

There was no consistent or significant ($p>0.05$) change in the SPAD values over the entire duration of treatment. At 16 dS/m salinity treatment, SPAD values were ranged from 33 to 42 in first month, whereas in five months the range was from 34 to 40 in *Atriplex lentiformis*

(Fig. 6). SPAD values were lower under high salinity treatments and there was no effect of irrigation and soil types but no such trend was apparent in *Medicago arborea*.

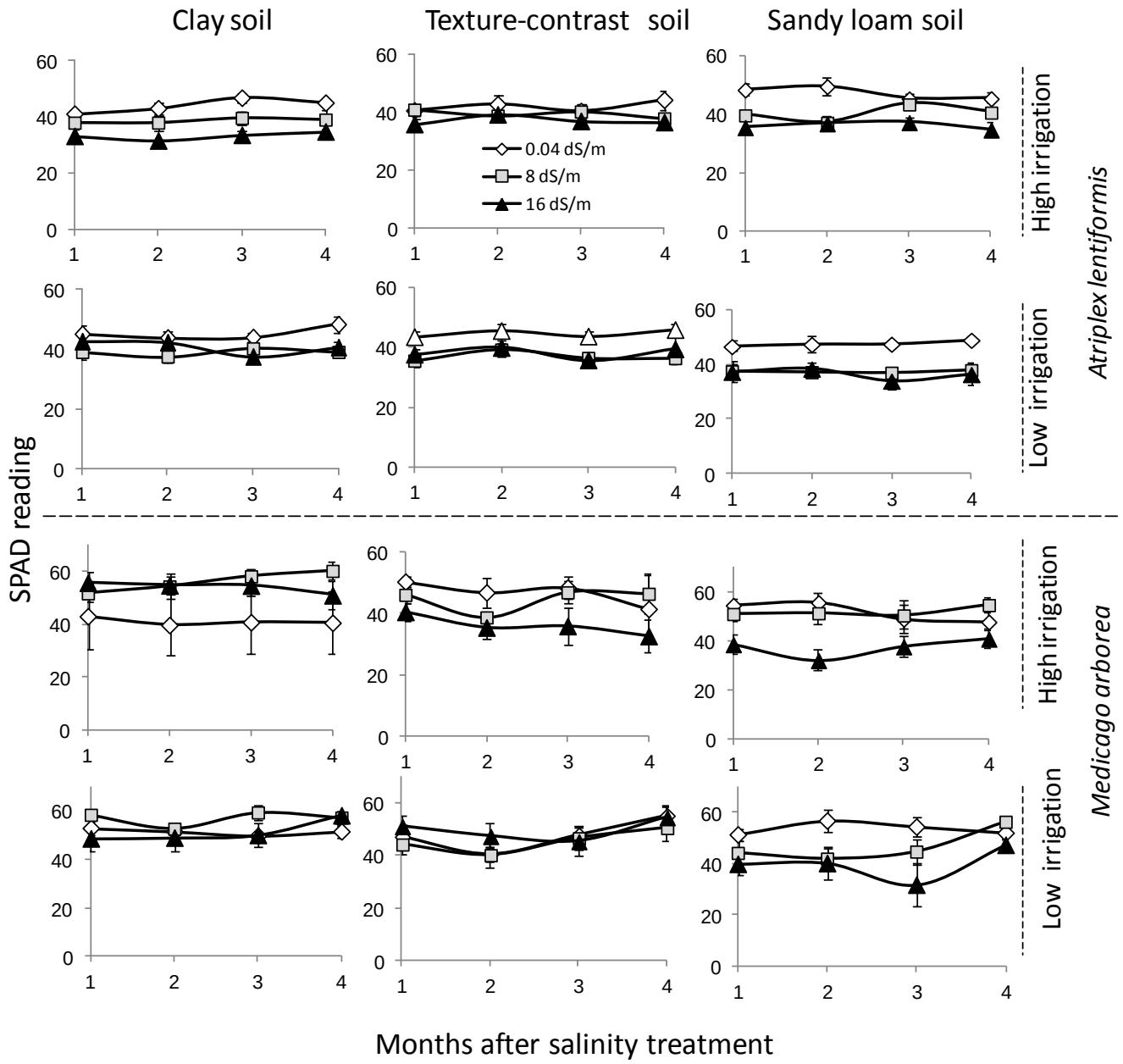

Fig. 6. Changes in the SPAD reading in *Atriplex lentiformis* and *Medicago arborea* over time. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml /pot/day, respectively.

4.3.7. Changes in the leaf sap osmolality

Osmolality values have gradually increased over the 5 months of treatment, regardless of the soil type (Fig. 7). This increase was much more pronounced in *Medicago arborea* compared with *Atriplex lentiformis*. If a comparison is made between the first and last month of treatment,

values did not change significantly ($p>0.05$) in *Atriplex lentiformis*. Regardless of the soil type, the irrigation salinity (ECw) had no impact on osmolality of *Atriplex* irrigated with the high irrigation rate but ECw had a significant ($p<0.05$) effect on *Medicago arborea,* especially in the sandy loam soil. In absolute terms, osmolality of *Medicago* plants was approximately 2-fold the osmolality of *Atriplex* plants (Fig. 7). In *Atriplex*, osmolality was higher in the sandy loam soil grown plants but in *Medicago* there was no difference between clay and sandy soil grown plants.

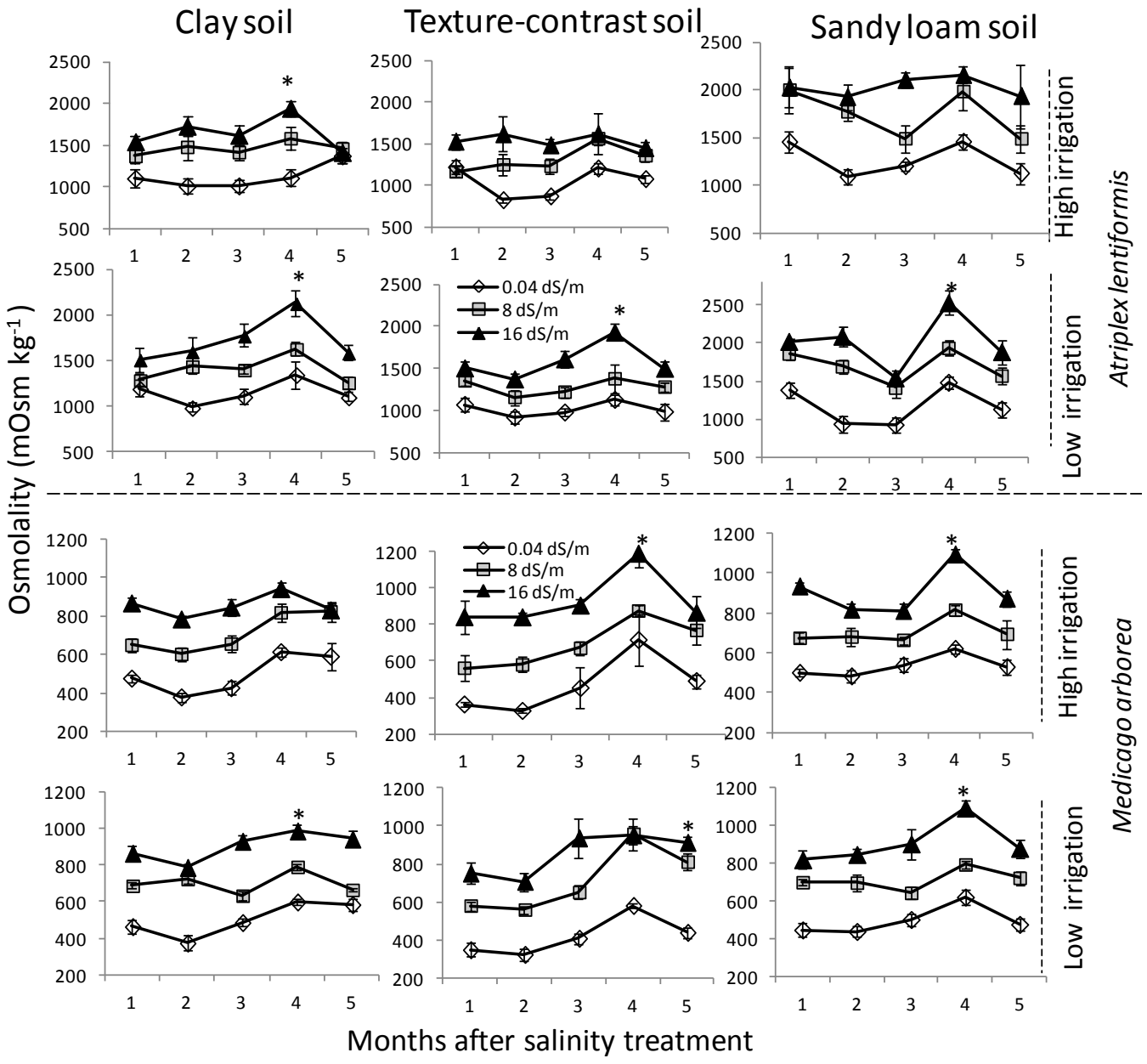

Fig. 7. Changes in leaf sap osmolality of *Atriplex lentiformis* and *Medicago arborea* plants over time. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml /pot/day, respectively. * indicates significant difference ($p<0.05$) of osmolality values between first and fifth month measurement within salinity treatment.

4.3.8. Changes in stomatal conductance (g_s)

A decline in stomatal conductance (g_s) was observed in both *Atriplex lentiformis* and *Medicago arborea* plants as the stress progressed. This decline was much more pronounced for *Medicago* compared with *Atriplex* (Fig. 8). Comparing data for clay soil, the g_s values in *Atriplex* declined 1.4-fold (from 33.5 ± 4.1 $mmolm^{-2}s^{-1}$ after 1 months of treatment to 23.5 ± 3.5 $mmolm^{-2}s^{-1}$. after 4 months of treatment), while in *Medicago* this decline was 2.8 fold (from 52.6 ± 4.1 to 18.2 ± 2.7 $mmolm^{-2}s^{-1}$ respectively). The amount of salt in the irrigation water had no significant (at $p<0.05$) impact on g_s values in *Atriplex* at any time point but were reduced dramatically (2.5 to 4 fold, depending on soil type and time) in *Medicago*. In both species, time-dependent g_s decline was more pronounced in the clay and texture-contrast soils and less pronounced in the sandy loam soil (Fig. 8). In *Atripex*, g_s values were largely independent on the soil type and irrigation rates (Fig. 8) while in *Medicago* they decline in a clearly pronounced sequence g_s clay > g_s texture-contrast > g_s sand. No significant ($p<0.05$) effect of irrigation was found for any soil type or species.

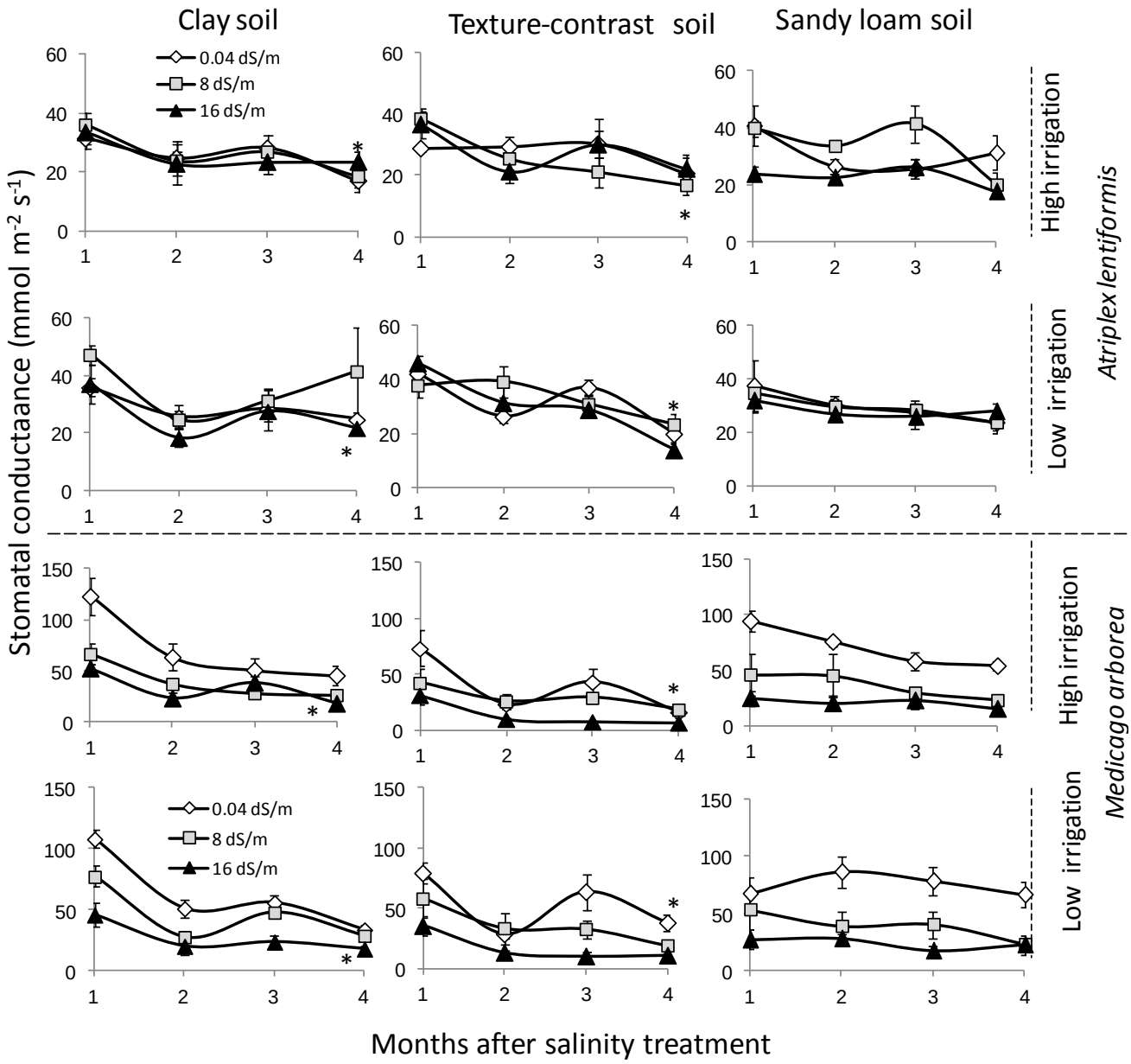

Fig. 8. Changes in the stomatal conductance (g_s) in *Atriplex lentiformis* and *Medicago arborea* over time. Values are mean ± S.E. (n = 5). Low irrigation and high irrigation means water was applied at 340 and 680 ml/pot/day, respectively. * indicates significant difference ($p<0.05$) of g_s values between first and fourth month measurement within salinity treatment.

4.4. Discussion

4.4.1. Soil texture influences salt accumulation in the soil

A marked increased in soil salinity in the clay dominated soil from 1 dS/m to the level of salinity concentration of the irrigation water applied within short period of time (5 months) has raised the question of the sustainability of the commercial practice of saline water irrigation in the long term. However, a 2 fold accumulation rate in the low irrigation rate indicated that at high irrigation rate salt may have been leached out through the soil. It needs to be kept in mind that this was a pot trial and excess water was allowed to drain easily out of the base of the pot. However under real field conditions high irrigation rates may create a waterlogging problem, water table rise or subsoil salinisation and plants have to deal with all or any of these stresses. Certainly a combination of waterlogging and salinity is usually highly unfavourable (Barrett-Lennard, 2003) as it results in reduced energy availability (Pottosin and Shabala, 2014) and disturbance to cytosolic ionic homeostasis and, specifically, potassium (Barret-Lennard and Shabala, 2013) caused by substantial membrane depolarization. On the other hand, Fig. 1 shows that sandy loam soil had 2.5 fold less ECse than the clay soil for the same treatment, and at 16 dS/m sandy soil average ECse was 50 % lower than the irrigation water (ECw). This may be related to the soil textures as water drains more easily in the coarser textured soils (Brady and Weil, 2009). It can be supported by the increasing leachate EC value in sandy loam soil with time (Fig. 2). This suggests that ions are easily leached out from the sandy loam soil. The reason for high salinity in clay soil may be due to the greater surface area of clay particles and the higher micro-porosity where soluble ions and exchangeable cations of saline water can attach and reside (Miller and Donahue, 1995, Ragab et al., 2008). At the same time, in sandy soils there is neither many negatively charged clay particles capable of holding the cations such as Na^+ and K^+, nor the high amount of micro-porosity and hence less salt accumulates. Moreover, the rate and the extent of salt movement in the soil correlated to solubility of salt, water quantity and soil permeability (Tedeschi and Dell'Aquila, 2005). This explains the reason for the lower ECse values of soils that was irrigated at the higher irrigation rates. In the clay soil, highly soluble NaCl salt may have been easily adsorbed and retained in the soil and thus did not leach out due to their low permeability and higher micro-porosity (Brady and Weil, 2009). Therefore, from soil salinity perspective, it indicates that coarse texture topsoils or root zones are better for the saline water irrigation. However, plants grown in the sandy soil will need nutrient supplements as nutrient holding capacity of such coarse textured soils is very low (Brady and Weil, 2009).

Similarly, soil pH was found increased due to the application of saline water. The sandy loam soil was more acidic compared to the clay dominated soil (Fig. 3) which may affect nutrient uptake by the plants (Marschner, 1995). Of specific importance is K^+ availability. Soil acidity is known to exacerbate K^+ deficiency in plants (Brady and Weil, 2009). This is clearly reflected in our leaf K^+ data (Fig. 5). Indeed, if one compares leaf sap K^+ content in *Medicago arborea* plants grown in clay and sandy loam soil after 1 month of salinity treatment at 8 dS/m irrigation, the difference is about 2-fold (153.9 ± 22.7 vs 80.9 ± 11.6 mM, respectively). Potassium leaching from the sandy soil may also explain a much sharper decline in leaf K^+ concentration measured in sand-grown plants (Fig. 5). In the texture-contrast soil, pH of sandy top (0 – 15 cm depth) was lower than the clay soil (15 – 30 cm) within the pot. This increase in pH values could be attributed due to the increase in Na^+ in the soil and the displacement of both H^+ and Ca^{2+} ions from the soil. For texture-contrast soils any ion displacement or leaching from the sandy loam top layer may become adsorbed in clay portion and hence pH value of 15 – 30 cm soil depth increased.

4.4.2. Kinetics of leaf Na^+ and K^+ accumulation is qualitatively different between *Atriplex lentifromis* and *Medicago arborea*

Control of xylem loading of Na^+ has been repeatedly reported as a key component of salinity stress tolerance in halophytes (Shabala, 2013; Shabala et al., 2013, 2014) and glycophytes (Munns and Tester, 2008; Shabala et al., 2010; Bose et al., 2014b). This is a highly dynamic process, and it was argued (Shabala, 2013) that the "ideal" scenario for a plant would be to quickly send the amount of Na^+ to the shoot required to rapidly achieve full osmotic adjustment and maintain the normal growth rate (hence, no yield penalties). Once this is achieved, it would be better for a plant to reduce the rate of xylem Na^+ loading to the absolute minimum required for driving cell turgor in newly growing tissues. This seems to be exactly the case here. In *Atriplex*, leaf Na^+ concentration remained constant over the entire duration of experiment (5 months) regardless of EC_w and irrigation levels, while in *Medicago arborea* a continuous accumulation of Na^+ in leaves was observed (Fig. 4; Table 1). This point out a very efficient mechanism of xylem Na^+ loading in *Atriplex*.

The specific ionic mechanisms beyond more efficient control of Na^+ loading in *Atriplex* remain the subject of a further investigation. Two aspects should be discussed here. The first one is the molecular identity of the ionic mechanisms mediating such loading. It was argued that xylem Na^+ loading may be a thermodynamically active process that needs energy to pump Na^+

into the xylem stream (De Boer and Volkov 2003; Shabala and Mackay, 2011; Shabala, 2013). This seems to be the case for the *Atriplex*, where the reported xylem Na^+ concentrations are 30 to 50 mM (Rozema et al., 1981) and the membrane potential of xylem parenchyma cells range between -130 and -140 mV (Anderson et al., 1977). Under such condition, a thermodynamically-passive (e.g. channel mediated) Na^+ loading will require cytosolic Na^+ concentrations in the parenchyma cells reaching a molar level, which is highly unlikely (see Munns and Tester, 2008 for arguments), with the highest cytosolic Na^+ values reported in the literature being 230 mM (Hajibagheri et al., 1987). This rules out the possible involvement of parenchyma-based ion channels, at least under conditions of elevated xylem Na^+ content and suggests that some (secondary) active transport system is involved. The most likely candidate is SOS1 Na^+/H^+ exchanger. In glycophytes, SOS1 is preferentially expressed at the xylem symplast boundary of roots (Shi et al., 2002), and it is also reported that SOS1 gene homologues have been cloned from several halophytes species (Maughan et al., 2009; Cosentino et al., 2010; Yadav et al., 2012). Notably, SOS1 activity was described to be inducible by saline condition, in both halophytes (Oh et al., 2010; Cosentino et al., 2010) and glycophytes (Shi et al., 2002).

Another possible candidate gene that has recently emerged as potential mediator of xylem Na^+ loading is a CCC (chloride-cation) co-transporter. CCC proteins are secondary active transporters that manage the movement of Cl^- (tightly coupled to that of K^+ and/or Na^+) across the plasmalemma (Haas, 1989) and these transporters have been found in both animal (Delpire and Mount, 2002) and plant (Colmenero-Flores et al., 2007) tissues. The GUS staining localised CCC expression to xylem parenchyma cells (Colmenero-Flores et al., 2007) and, as argued by Shabala (2013) the highly negative membrane potential will make Cl^- movement from the xylem parenchyma into the xylem thermodynamically passive, so can be used as a driving force to move Na^+ against the electrochemical potential and load it into the xylem.

The second important question is what regulates activity of either SOS1 or CCC (or both) transporters. Is this regulation transcriptional or post-translational? Are any of the stress-induced factors may be involved? It is known that the activity of xylem parenchyma-based ion channels is regulated by various factors such as polyamines (Zhao et al., 2007), apoplastic pH (Lacombe et al., 2000) and ABA (Pilot et al., 2003). All these factors are known to be modulated under saline environments. It remains therefore to be answered if any of these (or some other) factors may modify activity of either SOS1 or CCC transporters.

The above scenario observed in *Atriplex lentiformis* is not applicable to *Medicago arborea*. *Medicago* is a glycophyte plant and, thus, tries to minimise the Na^+ and Cl^- uptake and its delivery to the shoot. While it may be capable to do it for some time, five months of exposure

to salinity results in a progressive accumulation of Na^+ in *Medicago* leaves. From this point of view, our data reported here are consistent with the report by Bose et al., (2014a) on kinetics of xylem Na^+ loading and accumulation in pea, where plants were capable of preventing Na^+ loading for some time but failed to do it after a few weeks. At the same time, due to the lack of efficient tissue tolerance mechanism in the leaf, it is thought that excessive salt in *Medicago arborea* may have been accumulating in the cytoplasm that inhibits enzyme activities (Munns, 2000). It should be commented that the overall Na^+ concentration in *Medicago* was 182 mM while in *Atriplex* it was about 3-fold higher (541 mM) and yet *Atriplex* performance was not affected by salinity (see Chapter 3) while *Medicago* plants were dying. Taken together these finding strongly suggest that kinetics of xylem Na^+ loading and its sequestration in shoot vacuoles are absolutely essential in plant salt tolerance. *Atriplex* plants are much superior in each of these components, while *Medicago* plants fail to control both these processes.

4.4.3. Better K^+ retention explains superior performance of *Atriplex lentiformis* plants

Leaf K^+ concentration in *Atriplex lentiformis* was steady in the clay soil, while in *Medicago arborea* it showed a progressive decline (Fig 5; Table 1). Overall, the absolute leaf K^+ content in *Medicago arborea* was 2.2 fold lower than that of equivalent treatments in *Atriplex lentiformis* (Fig. 5). Thus, the above differences could not be attributed to soil factors but have to be explained by biological factors.

Under saline conditions, massive K^+ efflux was measured from roots of many species including wheat (Cuin et al., 2008, 2009; Wu et al., 2014), barley (Chen et al., 2005, 2007; Wu et al., 2015), lucerne (Smethurst et al., 2008), Arabidopsis (Shabala et al., 2005, 2006), poplar (Sun et al., 2009) and some other species. Experiments with the loss of function Arabidopsis mutants revealed that in most cases this efflux results from the NaCl-induced depolarization of root plasma membrane potential that results in activation of the outward-rectifying depolarization-activated GORK channels (Shabala and Cuin, 2008). Root K^+ retention ability under saline conditions has also correlated strongly with activity of H^+-ATPase and, therefore, the plant's ability to maintain highly negative membrane potential (Chen et al., 2007; Bose et al., 2015). For example, steady-state membrane potential values in *Atriplex* and *Chenopodium quinoa* (another halophyte species) plants were 15 to 20 mV more negative compared with Arabidopsis (Bose et al., 2015). It is therefore predicted that *Medicago* plants are not capable of maintaining membrane potential above the E_k (Nernst potential for K) values and, thus,

preventing NaCl-induced K^+ leakage via GORK channels. This may explain the gradual decline in plant K^+ content over the time.

A stronger decline in shoot K^+ content was observed in both plants grown in sandy loam (3.2-fold reduction over the 5-month period at 16 dS/m EC_w salinity for *Medicago arborea*) (Fig. 5 and Table 1). This may be attributed to the difference in the soil buffering capacity (Brady and Weil, 2009) and gradual K^+ leaching from pots. In addition, high levels of Na^+ in the soil solutions outcompetes other nutrient cations for uptake (Grattan and Grieve, 1999; Rengasamy et al., 2003) and causes K^+ desorption and leaching (Bartal et al., 1991; Jalali and Merrikhpour, 2008). The intracellular K^+ homeostasis is essential for many metabolic functions in cell such as turgor maintenance, enzyme activation and protein synthesis (Shabala 2003; Dreyer and Uozum 2011). If cytosolic K^+ pool is depleted and plant is not able to replenish it by any means, then the cell would die (Bose et al., 2014b).

4.4.4. *Atriplex* species is more efficient in achieving osmotic adjustment and controlling stomata aperture over the time

One of the hallmarks of halophytes is their ability to use inorganic ions such as Na^+, Cl^- and K^+ for osmotic adjustment under saline conditions (Shabala and Mackay 2011; Flowers and Colmer, 2008). This is further illustrated in this work by osmolality data. In *Atriplex lentiformis,* leaf sap osmolality was constant and did not change over the time (Fig. 7). When the relative contribution of inorganic and organic osmolytes towards leaf sap osmolality was calculated (Table 2), it was found that inorganic osmolytes (Na^+, Cl^- and K^+) had made the major contribution towards plant osmotic adjustment, making 97 ± 1% of total osmolality in clay and 85 ± 1% in sand. In *Medicago,* the role of inorganic osmolytes in osmotic adjustment was much smaller (71 ± 1% and 70 ± 8%, respectively) (Table 2). This indicates that organic solutes (such as sugars, amino acids, proline and quaternary amines) provided considerable contribution to turgor maintenance in *M. arborea.* However, this comes at a high carbon cost, as a significant amount of phytosynthates need to be used for *de novo* synthesis of these compatible solutes. Thus, a need to redirect a substantial part of the photosynthates' pool towards compatible solute production would compromise plant growth and development (Shabala, 2013). Furthermore, stomatal conductance (g_s) in *M arborea* was dramatically reduced over the time (Fig. 8), thus affecting the overall carbohydrate production and energy availability. The most likely reason for this is a reduced K^+ availability. Indeed, changes in g_s values correlated strongly with changes in leaf sap K^+ (Table 3), and K^+ is known to be absolutely essential in control of stomata aperture

under adverse environmental conditions (Benlloch-Gonzalez et al., 2010; Jin et al., 2011; Marschner, 2012; Wang et al., 2013)

Table 2. The relative contribution of ionorganic (Na^+; K^+; Cl^-) and organic osmolytes towards the leaf osmolality in *Atriplex lentiformis* and *Medicago arborea* plants grown in different soil types under 16 ds/m salinity irrigation.

Time after salinity treatment (months)	Plant	Soil types	Na^+ (mOsm kg^{-1})	K^+ (mOsm kg^{-1})	Cl^- (mOsm kg^{-1})	Total inorganic ions (mOsm $kg^{-1)}$	Osmolality (mOsm kg^{-1})	Relative contribution of inorganic osmolytes, %
1	MA	C	131	118	379	628	876	72
		SL	231	112	306	650	935	70
	AL	C	588	182	737	1507	1546	97
		SL	742	239	730	1710	2025	84
5	MA	C	203	120	277	599	943	64
		SL	340	57	330	727	877	83
	AL	C	600	135	621	1357	1417	96
		SL	802	104	711	1617	1935	84

Abbreviations: MA, *Medicago arborea*; AL, *Atriplex lentiformis*, C, Clay; SL, Sandy loam

Table 3. The correlation between different variables measured in high salinity treatment (16 dS/m). Data were pooled from all treatments (regardless of soil types and irrigation levels) and correlation was done by using monthly interval data.

Species		gs	SPAD	EC_{leach}	Leaf Na^+	Leaf K^+	osmolality
Atriplex lentiformis	gs	1	0.05	-0.28*	-0.23	0.68*	-0.45*
	SPAD	0.05	1	-0.42*	0.17	-0.14	0.11
	EC_{leach}	-0.28*	-0.42*	1	0.06	-0.38*	0.47*
	Leaf Na^+	-0.23	0.17	0.06	1	-0.20	0.63**
	Leaf K^+	0.68**	-0.14	-0.38*	-0.20	1	-0.38*
	Osmolality	-0.45*	0.11	0.47*	0.63**	-0.38*	1
Medicago arborea	gs	1	0.26*	0.32*	-0.78**	0.76**	-0.39*
	SPAD	0.26*	1	0.12	-0.09	0.21	0.16
	EC_{leach}	0.32*	0.12	1	-0.27*	0.60**	-0.13
	Leaf Na^+	-0.78**	-0.09	-0.27*	1	-0.77**	0.53**
	Leaf K^+	0.76**	0.21	0.60**	-0.77**	1	-0.18
	Osmolality	-0.39*	0.16	-0.14	0.53**	-0.18	1

Notes: significant at the *P< 0.01 level (2-tailed); * P< 0.05 levels (2 -tailed). Abbreviations: gs; stomatal conductance, Leaf Na+: leaf sap Na+ concentration, Leaf K+: leaf sap K+ concentration, Osmolality: leaf sap osmolality; SPAD: relative chlorophyll content, ECleach: Leachate electrical conductivity.

4.5. Conclusion

From the data above, it can be concluded that *Medicago arborea* plants grown under saline conditions showed the following characteristics: (1) strong reliance on organic osmolytes (hence, associated carbon costs) for osmotic adjustment; (2) poor K^+ retention that compromised stomata opening; (3) inability to prevent xylem Na^+ loading; and (4) poor shoot tissue tolerance, most likely due to inability to provide efficient Na^+ sequestration in vacuoles. On the other hand, *Atriplex lentiformis* showed an excellent ability of preventing Na^+ loading into the xylem stream and less reliance on organic osmolytes to deal with saline condition. In terms of soil health under saline irrigation, lower soil ECse at high irrigation rate suggests that salt may be leaching out due to high volume of water applied. Also, lower ECse of sandy loam soil compared to clay soil ECse indicates that soil texture can be one of the key determining factors in saline agriculture.

Chapter 5
Halophytes as a possible alternative to desalination plants: prospects of recycling saline wastewater during Coal Seam Gas operations
Suresh Panta, Peter Lane, Richard Doyle, Marcus Hardie, Gabriel Haros, and Sergey Shabala

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Chapter 6

Changes in soil chemical properties of a sodic texture-contrast soil (Brown Sodosol) after irrigation with saline water*

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Abstract

The concept of saline agriculture when crops are irrigated with low quality saline water is gaining momentum but the environmental impact of this practice remains debatable. In this work selected soil chemical properties to a depth of >2 m were assessed following 2.5 years of application of saline irrigation to a sodic texture-contrast (Brown Sodosol) in southern Tasmania. Control plots were compared to irrigation treatments of 0.8 dS/m and 16 dS/m at rates ranging from 200 to 800 mm/year. Significant impacts included electrical conductivity of soil saturated paste (ECse) and Cl^- concentration increasing between 0 – 200 cm depth in plots irrigated with 16 dS/m water. However this increase in soil ECse was lower than the irrigation water salinity of 16 dS/m. Topsoil (0 – 10 cm) total organic carbon (TOC) was significantly reduced in the high salinity plots and was negatively correlated with Cl^- concentration and soil pH. In the upper 50 cm of the high salinity irrigated plots both the exchangeable Na^+ and its ratio to total base cations (ESP) were significantly increased and the individual exchangeable base cations (Ca^{2+}, Mg^{2+}, K^+) were significantly decreased. The Ca:Mg ratio increased in the topsoils under high salinity irrigation regimes despite the leaching of both cations indicating relative displacement of ions based on charge/hydrated radii ratios. In clayey subsoil (below 40 cm), exchangeable Ca^{2+} was found to accumulate under saline irrigation with high saline

water while Mg^{2+} and K^{+} were leached even deeper. These data support the concerns associated with application of high saline irrigation waters namely increased salinisation but they also show beneficial declines in both pH and ESP in the both alkaline and sodic subsoils. They also show the Na^{+} in the saline waters causes differential leaching of base cations from the rooting zone to deeper in the soil.

Keywords: Soil salinity; Soils sodicity; Soil chemical properties; Exchangeable cations.

6.1. Introduction

The availability of fresh quality water is increasingly limiting irrigated agricultural production in many parts of the world, particularly eastern Mediterranean countries and Australia (Ventura et al., 2014; Schacht and Marschner, 2015). In Australia, the distribution of available water resources across the continent is highly variable, which places Australia at the crossroads in terms of its ability to cope with water scarcity (Charters and Willams, 2006). According to the latest data, total water use for agricultural production in Australia was 11.6 million megalitres in 2013-2014, which represents a 3% decline in water use from 2012-2013 (Australian Bureau of Statistics, 2015). Increased shortage of water for agricultural production has led to the development of alternative water resources including reclaimed waste water, saline drainage water and industrial saline co-produced ground water. For example, the use of recycled water increased by 27% between 2012/2013 and 2013/2014 (Australian Bureau of Statistics, 2015). Waste water produced from Coal Seam Gas (CSG) extraction is another potential source of water for irrigated agriculture. In 2013, CSG produced approximately 18,500 megalitres of wastewater but with salinity levels typically up to 17 dS/m (10 g/L; IESC, 2014).

Shortage of freshwater (EC_w<0.8 dS/m) has led to the use of saline water for agricultural production in many parts of the world, especially in arid and semi-arid regions; for example, in the Minqin basin in northwest China (Huang et al., 2011) and in the Middle East and North Africa (Abou-Hadid, 2003; Stenhouse and Kijne, 2006). In these regions saline irrigation water is used to grow salt-tolerant crops and fodder, which helps reduce the pressure and dependency on fresh-water resources for domestic users

and conventional agriculture. In the last few decades, saline water (salinity levels typically in the range of 2 – 10 dS/m), including recycled wastewater and groundwater, has been used for agricultural production in water limited regions of USA, Israel, Tunisia, India and Egypt (Rhoades et al., 1992). In Australia, there are a number of case studies on the use of groundwater (with water salinity up to 3.5 dS/m) (Christen et al., 2002) and recycled water (1.3 dS/m) (Stevens et al., 2008) for agricultural production. About 1% of all terrestrial plants are classified as halophytes (Flowers et al., 2010) and therefore benefit from the presence of significant amounts of NaCl in the soil solution. Importantly, many of them can and are already used as alternative crop or forage species (Panta et al., 2014).

However, irrigation with saline water may lead to soil salinity and sodicity. As it is estimated that 7% of the world's total land (Munns, 2005) and 11% of the world's irrigated areas are already affected by some degree of salinisation (FAO, 2012), saline water, which can exacerbate this soil salinisation process and may cause irreversible damage in the environment, must be used with caution. The dangers are illustrated by the fact 2,000 – 4,000 ha/day of irrigated land is degraded by salinity in arid and semi arid regions (Shabala, 2013; Qadir et al., 2014), becoming unsuitable for traditional food crop production. In 2013, loss in crop production due to salt induced degradation of irrigated land was estimated at over US$27.3 billon (Qadir et al., 2014). Previous studies, undertaken both in Australia and elsewhere, on the impacts of saline wastewater on soil have provided some information on changes in soil salinity (Thompson 1991; Angin et al., 2005; Chen et al., 2013), sodicity (Mantell et al., 1985, Burrow et al., 2002; Surapaneni, 2002), soil aggregate stability (Schact et al., 2014, Tarchitzky et al., 2007) and hydraulic conductivity (Shainberg et al., 1981; Sharma and Manchanda, 1996; Halliwell et al., 2001) or to a lesser extent, on soil chemical properties (Rogers, 2002; Tedeshi and Dell' Aquila, 2005; Ganjegunte et al., 2005; Jalali et al., 2008 ; Biggs and Jiang, 2009). However, changes in soil chemistry due to saline water irrigation still remains poorly understood. An understanding of the effect of the application of saline water on the soil chemistry is necessary to avoid the serious nutritional disorders such as toxicity or deficiency of certain nutrients for the plant growth in such soils.

Although the use of saline water helps to ease the pressure on the shortage of fresh water for agricultural crop and forage production, consequent soil deterioration raises the question of the long term sustainable use of such waters in agriculture. Conversely, if use of saline water is prevented by specific state or national government regulation it raises questions over the increasing supply demands on freshwater to increase the food production by 70 % by 2050 to meet the food demand of the projected 9.3 billion population of the world (Tester and Langridge, 2010; Shabala, 2013). Therefore, a careful management of irrigation practices is necessary to avoid yield loss and land degradation. It is also notable that most of the salinisation issues related to saline irrigation water occur in arid and semiarid regions because of high evaporation and low precipitation rates in those regions (Ventura et al., 2014). In such environments annual rainfall is insufficient to leach out the accumulated salts from the root zone. But these effects may not be consistent in all soil types and environmental conditions. In this study, we investigated the effect of saline irrigation water (EC_w up to 16 dS/m and irrigation rates up to 800 mm/year) on the chemical properties of a texture-contrast (Brown Sodosol) soil in a region with an average annual rainfall of around 500 mm/year.

6.2. Materials and methods

6.2.1. Site description

The study site was located approximately 25 km NE of Hobart in the Coal River Valley, Tasmania, Australia. The soil consists of an aeolian derived fine sandy loam (greyish yellow brown – 10YR 4/2) A1 and A2 horizons 0 – 20 cm above a very deep mottled-sodic clay to sandy clay subsoil (Holz, 1993). The soil classifies as a Mottled-Subnatric Brown Sodosol according to Australian soil classification (Isbell, 2002), a Hyposodic Luvisol according to the World Reference Base (FAO/ISRIC/ISSS, 1998) and a Natrixeralfs in the US Soil Taxonomy (Soil Survey Staff, 2006). The site was formerly used for pasture and cereal production. The region is described as dry subhumid and warm area (Holz, 1993) with average annual rainfall of 469 mm and evaporation of 1314 mm, measured from 1981 – 2010 (Bureau of Meteorology, 2014). The soil chemical properties before initiation of experiment are given in Table 1.

Table 1. Initial selected soil chemical properties of the experimental site.

Depth (cm)	ECse (dS/m)	pH(1:5:: soil:water)	TOC (%)	Cl^- (mg/kg)	Exchangeable ions (cmol(+)/kg)				TEB (cmol(+)/kg)
					Ca^{2+}	Mg^{2+}	Na^+	K^+	
0-10	0.82	7.13	1.88	20.0	8.7	1.9	0.10	0.24	10.9
10-20	1.41	7.08	1.37	68.0	7.8	3.1	0.30	0.20	11.5
20-30	0.80	7.25	0.47	18.5	8.9	9.8	0.69	0.19	19.6
30-50	1.02	7.58	0.32	43.9	9.6	12.5	1.41	0.25	23.7
50-100	1.06	8.93	0.52	22.4	8.1	14.8	3.64	0.46	26.9
100-200	1.17	9.08	0.19	46.1	9.8	16.6	2.76	0.68	29.9
200-300	0.81	9.29	0.13	28.1	12.7	10.8	0.94	0.48	24.9

TOC: Total organic carbon, Cl^-: Chloride, ECse: Electrical conductivity of soil saturate paste extract, TEB: Total exchangeable bases.

6.2.2. Experimental design and plot layout

The experiment consisted of 9 treatments: three irrigation rates, 200, 500 and 800 mm/year which increased sequentially in a west to east direction, and three salt concentrations 0.8, 8.0 and 16 dS/m which increased in a south to north direction (as shown in Fig. 1).

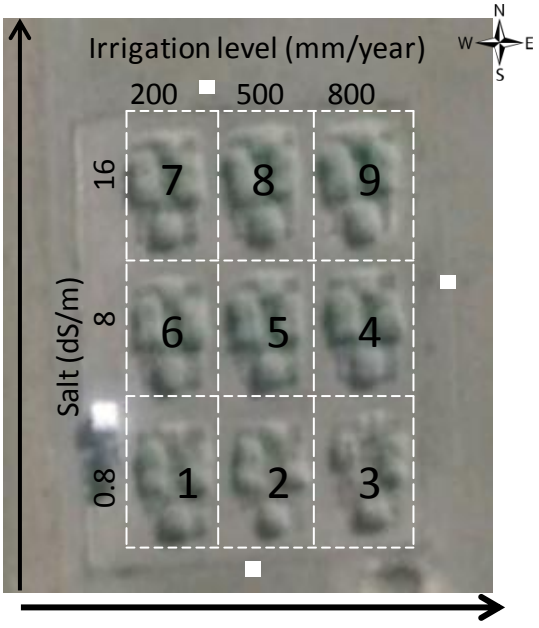

Fig. 1. Google earth aerial view of experimental site in 2015, where numbers 1 to 9 represent the experimental treatment plots and white boxes are the control plots outside the irrigation area.

Five plant species namely, *Atriplex lentiformis*, *Atriplex halimus*, *Medicago arborea*, *Medicago sativa*, and *Puccinellia ciliata* were grown to assess their performance, especially for biomass production and nutritional quality under different salinity and irrigation treatments. The plant growing procedures and the preliminary results were described in Panta et al. (2015). But in this paper we describe the effect of saline water on the soil chemical properties.

Soil sampling and analysis were conducted on samples collected from the 200 and 800 mm irrigation rate and 0.8 and 16 dS/m salt concentration treatment plots. The 200 mm irrigation rate represents normal deficit irrigation practice in the region, whilst 500 mm is commonly applied to crops such as lucerne and 800 mm represents 95% of the rainfall–evaporation deficit. Salt concentration of 0.8 dS/m was selected as it represents the average salt content of recycled domestic wastewater used in the Coal valley region, Tasmania (Table 2). The 8 and 16 dS/m treatments were selected as representing water salinity produced by the Coal Seam Gas (CGS) Industry that may be used for irrigation. Soil was also sampled from an unirrigated area adjacent to the experimental site as a control. All plots were irrigated on a weekly basis regardless of rainfall. Total irrigated volume per year and salt application over 30 months of the trials are presented in Table 3.

Table 2. Composition of irrigation water used for the experiment (waters samples were analysed in CSBP laboratory in Western Australia).

Particulars	Units	Water salinity treatments		
		0.8 (dS/m)	8.0 (dS/m	16 (dS/m)
Ammonium Nitrogen	mg/L	<0.10	<0.10	<0.10
Nitrate nitrogen	mg/L	11.54	11.64	11.54
Boron	mg/L	<0.05	<0.05	<0.05
Calcium	mg/L	22.44	22.04	22.30
Chloride	mg/L	147.80	2673.14	5796.5
Copper	mg/L	<0.05	<0.05	<0.05
Iron	mg/L	<0.05	<0.05	<0.05
Magnesium	mg/L	17.27	16.06	15.81
Manganese	mg/L	<0.05	<0.05	<0.05
Phosphorus	mg/L	4.33	4.10	3.99
Potassium	mg/L	14.91	15.02	16.66
Sodium	mg/L	86.8	1567	3622
Suplhur	mg/L	14.72	15.14	16.12
Zinc	mg/L	<0.05	<0.05	<0.05
pH		7.0	7.1	7.0
Electrical conductivity	dS/m	0.8	8	16
SAR		3.1	60.84	142.4

Table 3. Details of plots selected for soil analysis.

Treatment	Salinity level (dS/m)	Irrigation volume (mm/year)	Salt added kg/year	Cumulative salt added in 2.5 years (kg)	Selected plot for soil chemical analysis
Control	0	0	0	0	Control
P1	0.8	200	9	23	P1
P3	0.8	800	37	92	P3
P7	16	200	183	458	P7
P9	16	800	733	1833	P9

6.2.3. Soil sampling and analysis

Soil core sampling was restricted to the highest and lowest combinations of irrigation rate and salt concentration, Plot 1 (200 mm/yr at 0.8 dS/m), Plot 3 (800 mm/year at 0.8 dS/m), Plot 7 (200 mm/year at 16 dS/m) and Plot 9 (800 mm/year at 16 dS/m), plus control plots which received only rainfall (Table 3). Samples were collected, using a hydraulically driven 4.5 cm diameter push tube, in triplicate, in each plot, after 2.5 years of saline water irrigation (Fig. 2). Soil samples were subdivided into 0 – 10, 10 – 20, 20 – 30, 30 – 50, 50 – 100, 100 – 200, 200 – 300 cm sections to analyse the soil chemical properties with depth.

Soil pH was measured in distilled water (pH_{water}) and in 0.01M $CaCl_2$ solution (pH_{CaCl2}) at a ratio of one part of soil to five parts of distilled water as described in method 4A1 and 4B1 in Rayment and Higginson (1992). The saturated paste extract soil electrical conductivity (ECse), total organic carbon (TOC), chloride (Cl^-) and exchangeable cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) were determined by CSBP soil and plant analysis laboratory, Western Australia. Soil electrical conductivity (ECse) was measured from a saturated paste extract to enable direct correlation to plant stress. Air dried < 2 mm, 300 g of ground soil was mixed with distilled water until saturated and left overnight to equilibrate before centrifuging. Saturation paste extracts were collected and measured for electrical conductivity as described in Rayment and Higginson (1992). Total organic carbon (TOC) was determined by the Walkley-Black method using concentrated H_2SO_4 in the presence of dichromate solution with chromic ions produced was measured on a spectrophotometer at 600nm. Water soluble soil chloride (Cl^-) was measured in 1:5 soil: water extracts by Inductively Coupled Plasma (ICP) Spectroscopy as described in

Rayment and Lyons (2011). Exchangeable cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) were determined following alcohol (60% ethanol) prewash to remove soluble cations. Cations were extracted with 1 M NH_4Cl for 1 hour, and determined by Inductively Coupled Plasma (ICP) Spectroscopy 15A2 Rayment and Lyons (2011). Soil sodicity was calculated as exchangeable sodium percentage or ESP = (100 x Exchangeable Na) / Exchangeable (Ca^{2+} + Mg^{2+} + Na^+ + K^+). Total exchangeable bases (TEB) are the sum of all exchangeable base cations (Ca^{2+} + Mg^{2+} + Na^+ + K^+).

Spatial variation in soil apparent electromagnetic conductivity was mapped prior to irrigation in October 2011 and 24 months after irrigation commenced (April 2014) by using a hand held Geonic EMI38 in both the vertical and horizontal dipole on a 2 x 2 meter spacing (Fig. 3). Maps of apparent conductivity were produced in SURFER Version 8 employing default kriging settings and low level of contour smoothing.

Fig. 2. Soil sampling using hydraulically driven 4.5 cm diameter push tube.

Fig. 3. Equipment used for EMI survey, Geonomics EMI 38, and Garmin 12 XL GPS. (Courtesy from Marcus Hardie).

6.2.4. Statistical analysis

The difference between treatments were explored on a depth by depth basis using one way ANOVA with Tukey post hoc test in SPSS version 19. The relationship between individual variables was examined by simple linear correlations and regressions in Excel data analysis tools.

6.3. Results

6.3.1. Soil apparent conductivity

Prior to irrigation apparent conductivity varied between 0 to 45 mS/m across the field site. After irrigation, apparent conductivity increased in proportion to both salt concentration and irrigation volume. In Plot 9 (16 dS/m at 800 mm/year), apparent conductivity increased from approximately 30 – 40 mS/cm to 80 – 120 mS/cm following 24 month's irrigation (Fig. 4). Irrigation with 0.8 dS/m water did not appear to influence apparent conductivity, regardless of irrigation volume (Plots 1, 2 and 3). A notable

increase in apparent conductivity appeared to occur when irrigation water was applied at 500 or 800 mm/yr at 8 dS/m (Plot 4 and 5) or for all irrigation rates at 16 dS/m (Plots 7, 8 and 9).

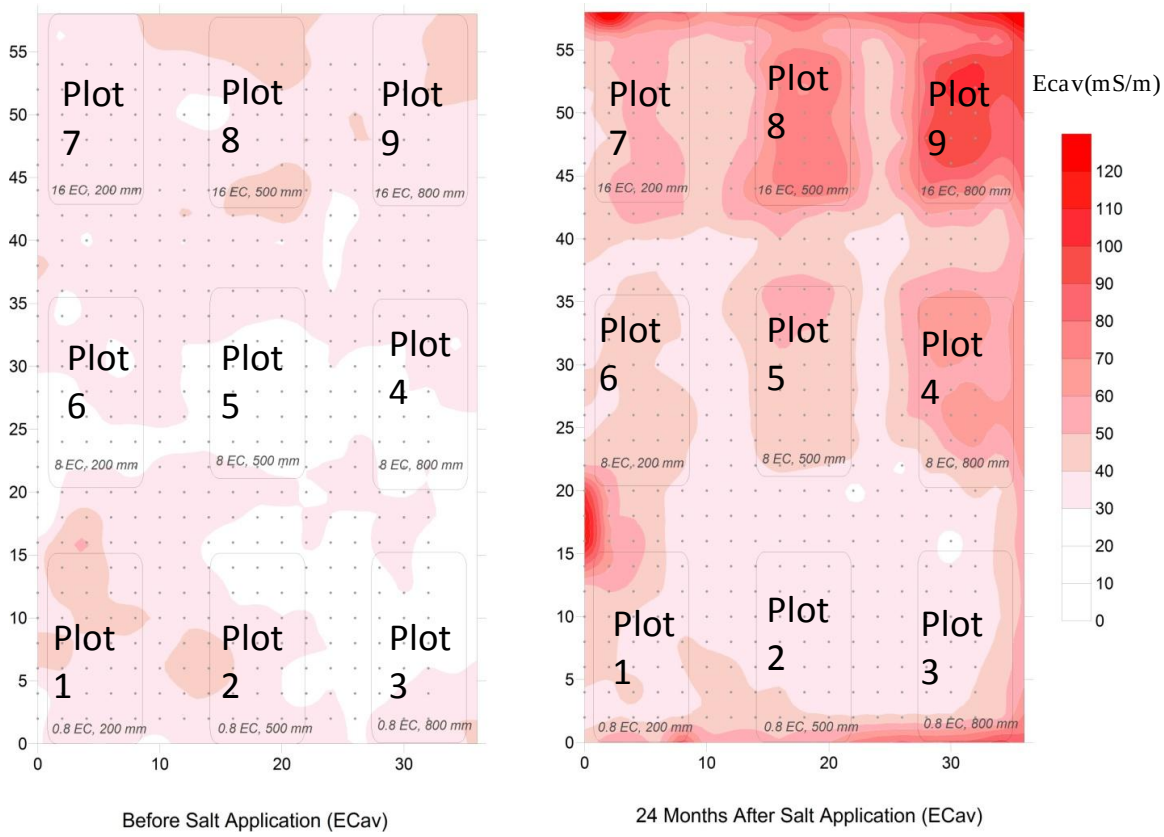

Fig. 4. Soil apparent conductivity in vertical dipole (ECav) before treatment and after 24 months of saline treatment. Where black dot represents data collection point on 2 x 2 m grid (conversion 100 mS/m = 1 dS/m).

6.3.2. Trends in soil properties

6.3.2.1. Soil pH

The active root zone for the species grown is within the top 50 cm; therefore the soil physical and chemical properties in this zone would have a major impact on plant nutrient acquisition and the overall performance. Both the soil pH trends (water and $CaCl_2$) with depth show an initial decline (10 – 30 cm) then a significant increase to a relatively constant alkaline pH (> 8) in the subsoils (>50 cm) . Irrigation at the highest rate (800 mm/yr) with highly saline water (16 dS/m) (Plot 9) significantly increased soil

pH in the topsoil (0 – 10 cm depth) relative to the control, whilst significantly reducing soil pH below 50 cm depth. At 20 – 40 cm depth difference in pH between plots was not significant ($p < 0.05$) (Fig. 5).

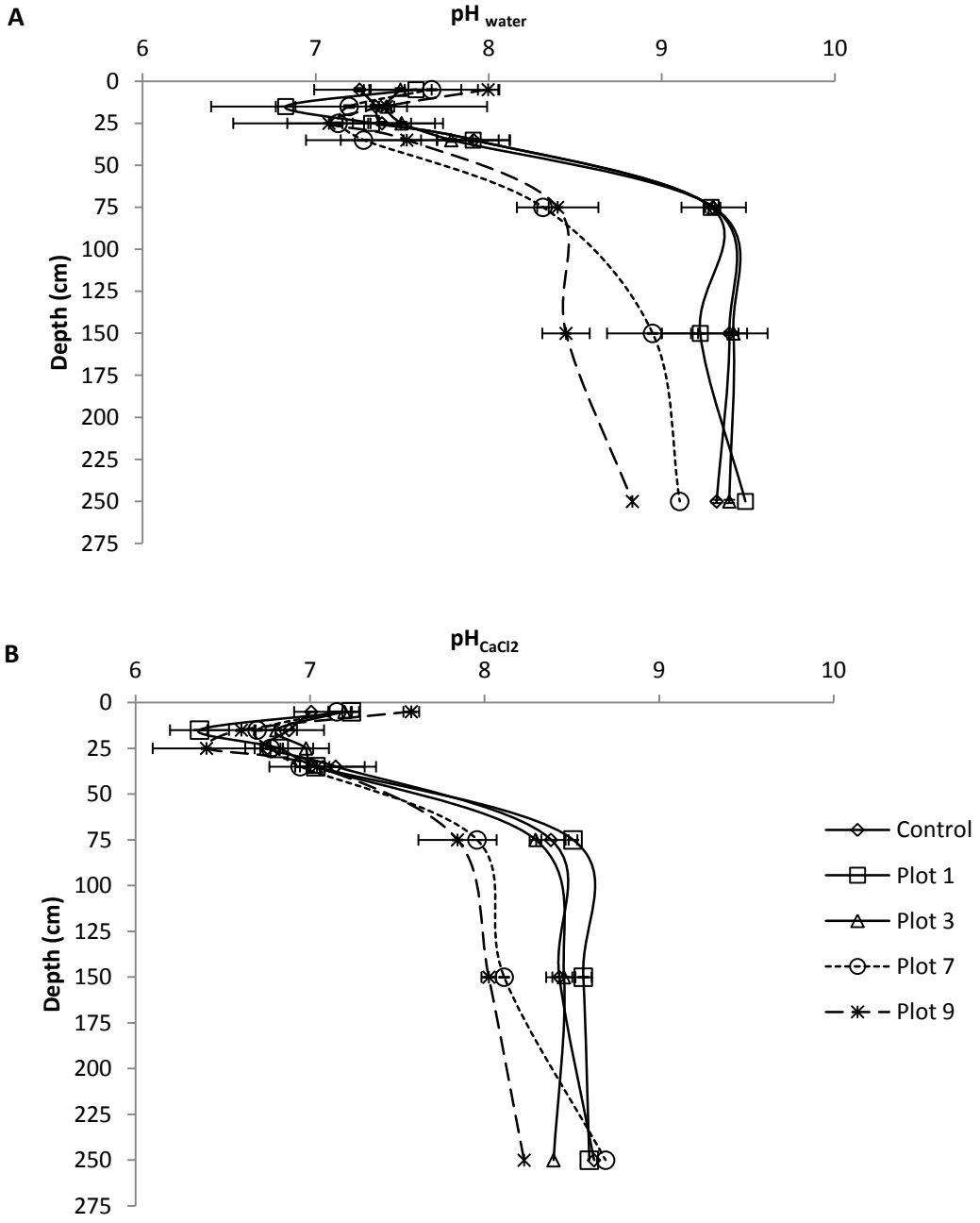

Fig. 5. Soil pH values of selected plots under different salt and irrigation regimes pH_{water} (A) and pH_{CaCl2} (B). Values are the mean ± S.E., where n = 3.

6.3.2.2. Soil electrical conductivity (ECse)

The soil ECse increased with increasing irrigation water salinity. Fig. 6 shows, following 2.5 years, irrigation with 0.8 dS/m water resulted in moderate increases (non significant) in soil salinity (< 2.7 dS/m) throughout the soil profile compared to the control. Irrigation with 16 dS/m water at volumes of both 200 mm/yr (Plot 7) and 800 mm/yr (Plot 9) significantly increased ECse at all depths. The soil ECse after 2.5 years of irrigation with 16 dS/m at 800 mm/yr was 20 fold higher than the control at 0 – 10 cm, 12 times higher at 75 cm depth and seven times higher at 250 cm depth.

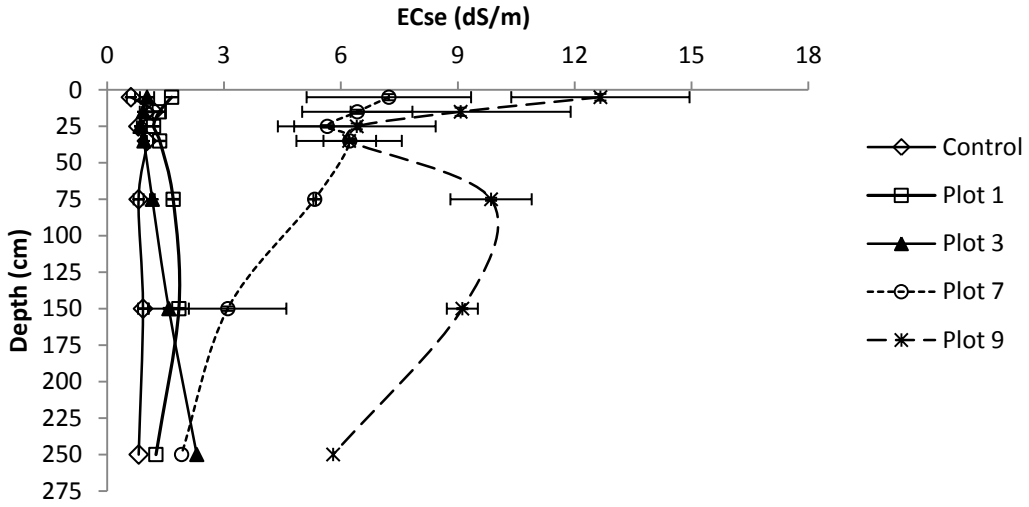

Fig. 6. Soil electrical conductivity of saturate paste extract (ECse) in selected experimental plots. Values are the mean ± S.E., where n = 3.

6.3.2.3. Total organic carbon (TOC)

Saline irrigation had minimal effect on soil organic carbon. At the 0 – 10 cm depth the TOC content of Plot 9 was significantly lower ($p<0.05$) than the other plots; however, below this depth no other significant differences in TOC existed (Fig. 7).

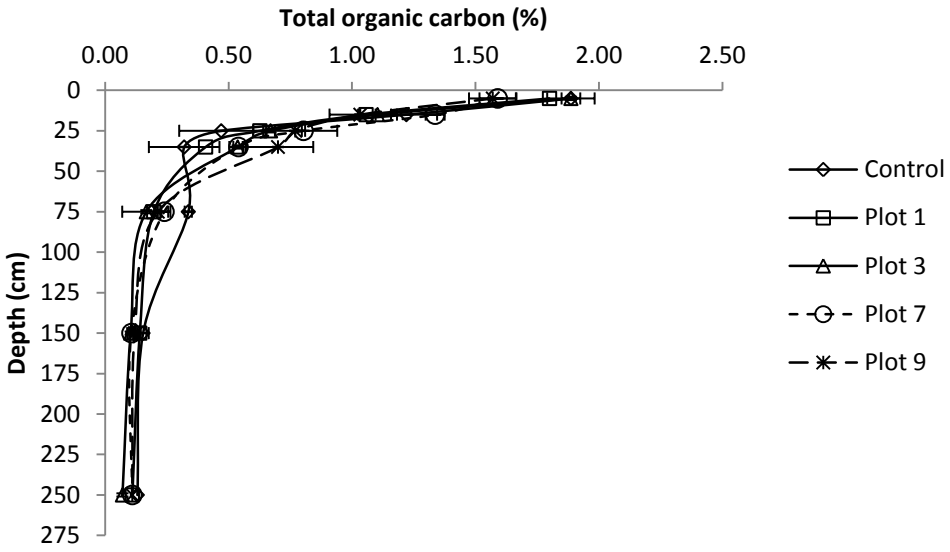

Fig. 7. Total organic carbon (TOC) in the soil of selected plots. Values are the mean ± S.E., where n = 3.

6.3.2.4. Exchangeable base cations

Saline irrigation tended to decrease exchangeable Ca^{2+} in the topsoil (0 – 30 cm) and increase exchangeable Ca^{2+} in the subsoils (50 – 200 cm depth), whilst saline irrigation tended to decrease the concentration of exchangeable Mg^{2+} in the upper 200 cm of the profile. In Plot 9 exchangeable Ca^{2+} was significantly lower than the other plots between 0 – 50 cm depth and higher than the other plots between 50 and 200 cm depth (Fig. 8). Exchangeable Mg^{2+} was consistently lower at all soil depths in the high salinity irrigated plots (7 and 9) than the plots irrigated with 0.8 dS/m water (Fig. 9). Similarly, exchangeable K^{+} concentration was significantly ($p<0.05$) lower above 200 cm in Plots 7 and 9 relative to that of the control (Fig. 10).

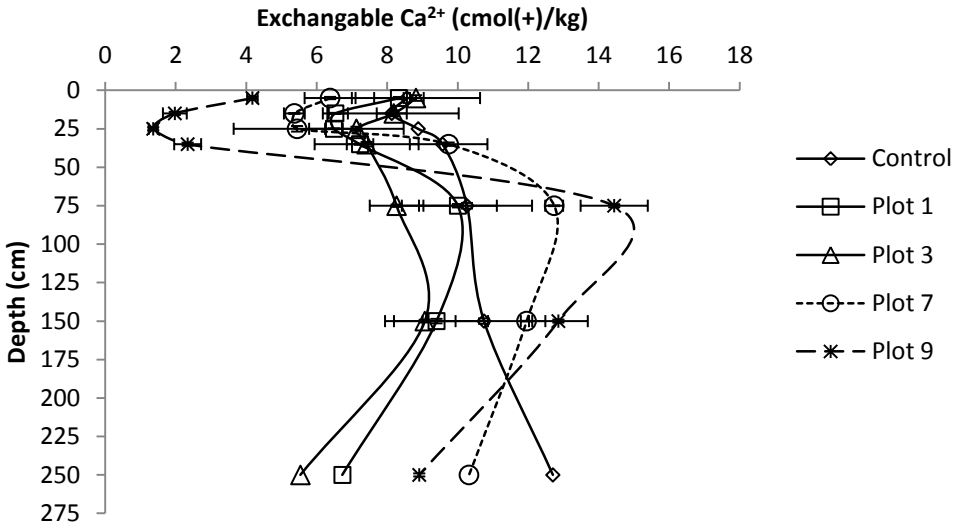

Fig. 8. Exchangeable calcium (Ca^{2+}) in the soil of selected plots. Values are the mean ± S.E., where n=3.

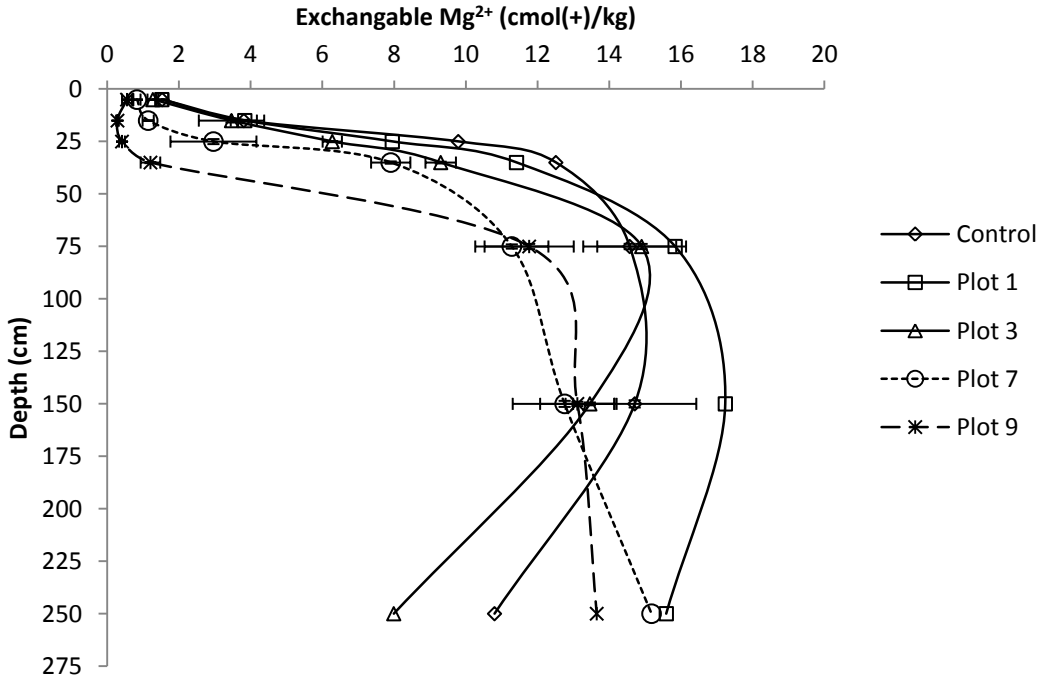

Fig. 9. Exchangeable magnesium (Mg^{2+}) in the soil of selected plots. Values are the mean ± S.E., where n = 3.

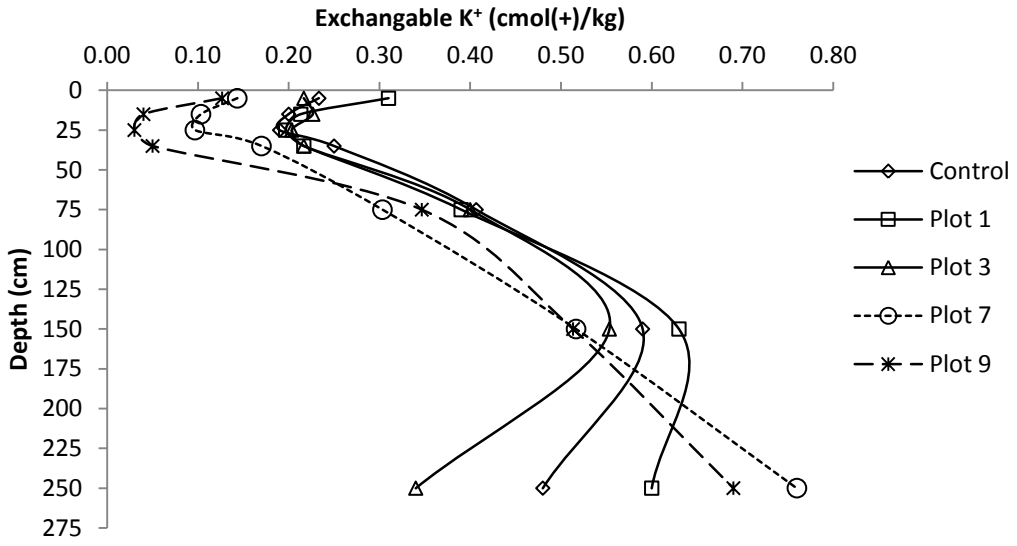

Fig. 10. Exchangeable potassium (K^+) in the soil of selected plots. Values are the mean ± SE, where n = 3.

Exchangeable Na^+ concentration differed markedly between treatments and depths in the soil (Fig.11). In the topsoil (0 – 10 cm depth) irrigation with highly saline water (Plots 7 and 9) significantly increased exchangeable Na^+ from 0.10 cmol(+)/kg in the control to 1.32 cmol(+)/kg in Plot 7 and 2.75 cmol(+)/kg in Plot 9. In the subsoil (<50 cm depth) exchangeable Na^+ concentrations were more variable but at 50 – 100 cm Na^+ was significantly lower in Plot 7 than all others, whereas at 100 - 200 cm Na^+ in Plot 9 was significantly lower than all others, whilst below 200 cm depth Na^+ concentration in Plots 1, 7 and 9 were higher than the Control plot.

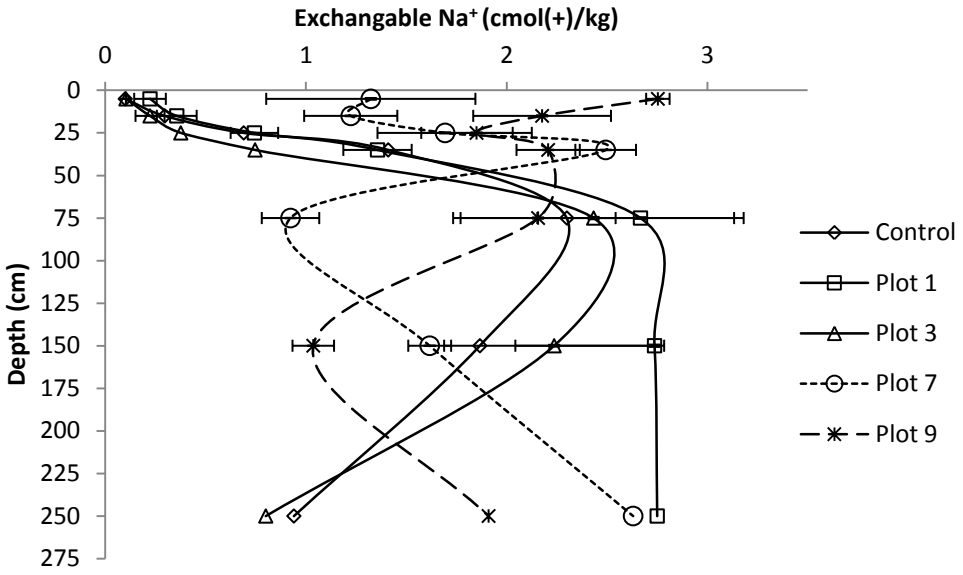

Fig. 11. Exchangeable sodium (Na^+) in the soil of selected plots. Values are the mean ± SE, where n=3.

6.3.2.5. Chloride concentration

Chloride concentrations in Plots 7 and 9 were significantly higher ($p<0.001$) at all soil depths compared to all other plots (Fig. 12). Topsoil Cl^- concentration in Plot 9 was 57, 10 and 17 times higher than the Control Plot, Plot 1 and Plot 3, respectively. In Plot 9, the Cl^- concentration in the topsoil at 0 – 10 cm depth was 1089 mg/kg which gradually decreased with depth to 50 cm depth and again increased significantly. In Plot 7, Cl^- concentration gradually increased to a depth of 50 cm from 778 mg/kg at the surface soil (0 – 10 cm depth) to 1293 mg/kg at 30 – 50 cm depth. Overall, Cl^- concentration was substantially higher in highly saline water (16 dS/m) irrigated plots at all depth compared to the control and 0.8 dS/m irrigated plots.

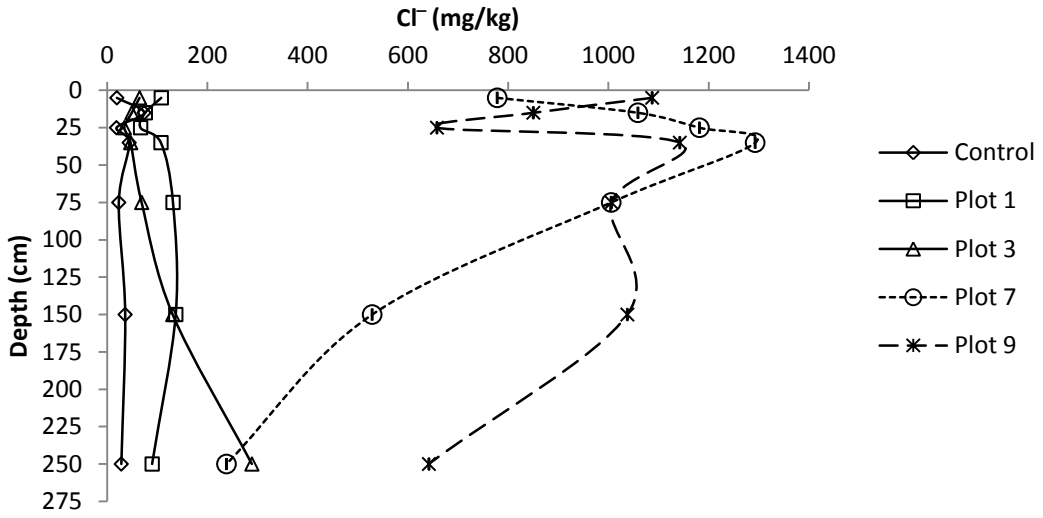

Fig. 12. Chloride (Cl^-) concentrations in the soil of selected plots. Values are the mean ± S.E., where n = 3.

6.3.2.6. Exchangeable sodium percentage (ESP)

The exchangeable sodium percentage (ESP) values of Plots 7 and 9 were significantly higher ($p<0.001$) between 0 – 50 cm depth compared to the Control, Plot 1 and Plot 3. In the topsoil (0 – 10 cm depth) ESP was 16 times higher for Plot 7 and 37 times higher for Plot 9 than the Control Plot (Fig. 13).

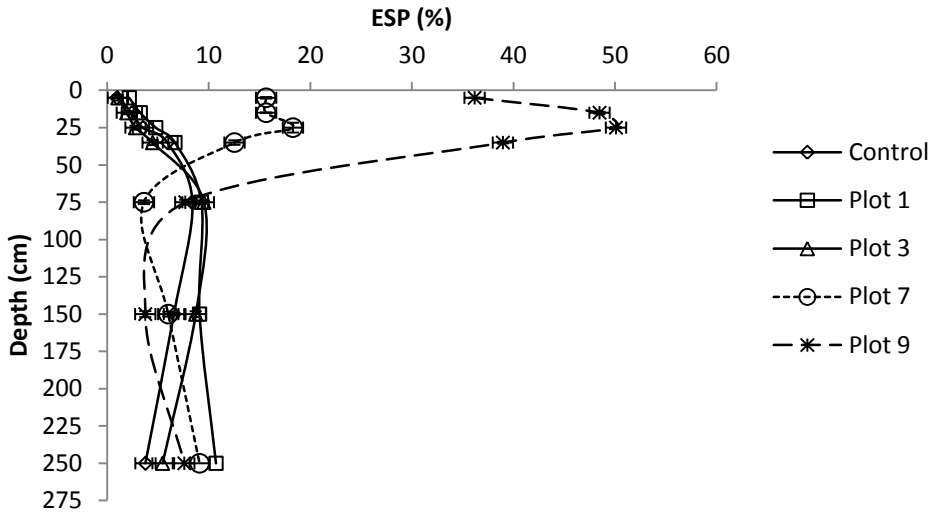

Fig. 13. Exchangeable sodium percentage (ESP) in the soil of selected plots. Values are the mean ± S.E., where n = 3.

6.3.2.7. Ca^{2+}/Mg^{2+} ratio

Saline irrigation resulted in proportionally greater accumulation of Ca^{2+} relative to that of Mg^{2+} at 0 – 50 cm depth. The significantly higher ($p<0.001$) Ca/Mg ratio was observed in Plot 9 and Plot 7 to a depth of 50 cm in the soil profile and its ratio was lower between 75 and 150 cm compared to other plots (Fig. 14). But the Ca^{2+}/Mg^{2+} values in Plot 1, Plot 3 and the Control were very similar at all depths and were not significantly different from each other.

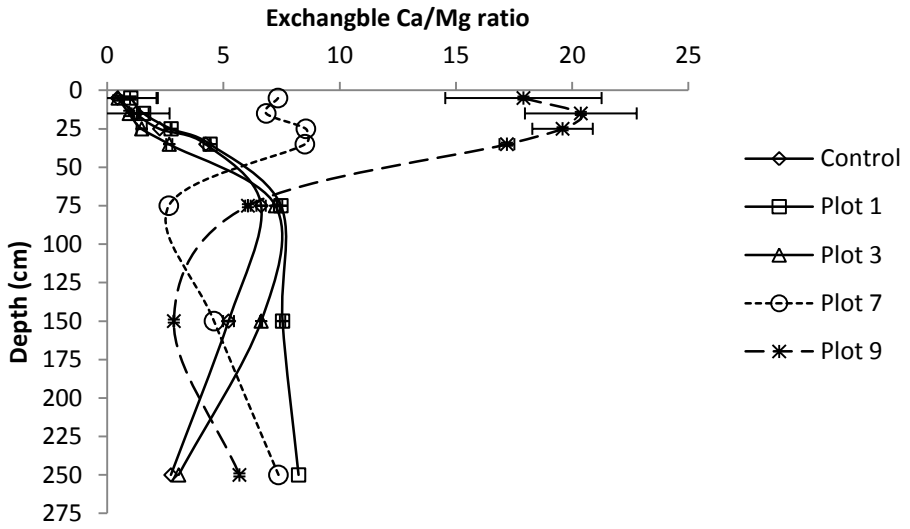

Fig. 14. Exchangeable Ca^{2+}/Mg^{2+} ratio in the soil of selected plots. Values are the mean ± S.E., where n = 3.

6.3.2.8. Total exchangeable bases (TEB)

Saline irrigation tended to decrease TEB in the topsoil (<50 cm) but with no clear trends in the subsoils. The TEB at 0 – 50 cm in Plots 7 and 9 was lower compared to low saline water irrigated in Plots 1, 3 and the Control Plots. In the topsoil (<30 cm depth) irrigation with high levels of saline water (Plot 9) significantly ($p<0.001$) deceased TEB by a factor of about 2 and 3 times that of the non irrigated control. There was no apparent difference in TEB between plots below 50 cm depth (Fig. 15).

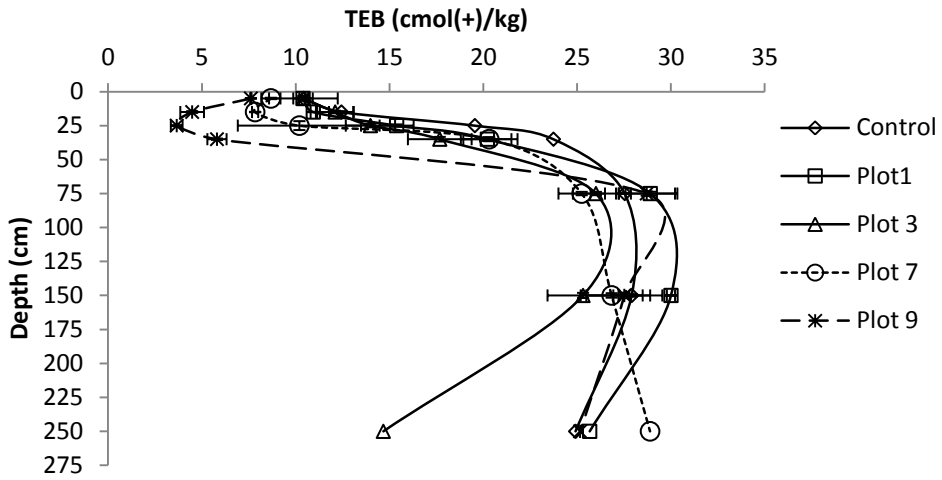

Fig. 15. Total exchangeable bases (TEB) in the soil of selected plots. Values are the mean ± S.E., where n = 3.

6.3.3. Relationships between selected chemical properties

Significant correlations ($p<0.05$) were observed among some selected chemical properties. The obvious highly significant positive correlation between ECse with Cl^- ($p<0.001$) there were also positive correlations between ECse and pH and exchangeable Na^+ (both $p<0.001$). However, total organic carbon (TOC) was significantly negatively correlated with both ECse and Cl^- ($p<0.001$) and also pH ($p<0.05$, Fig. 16).

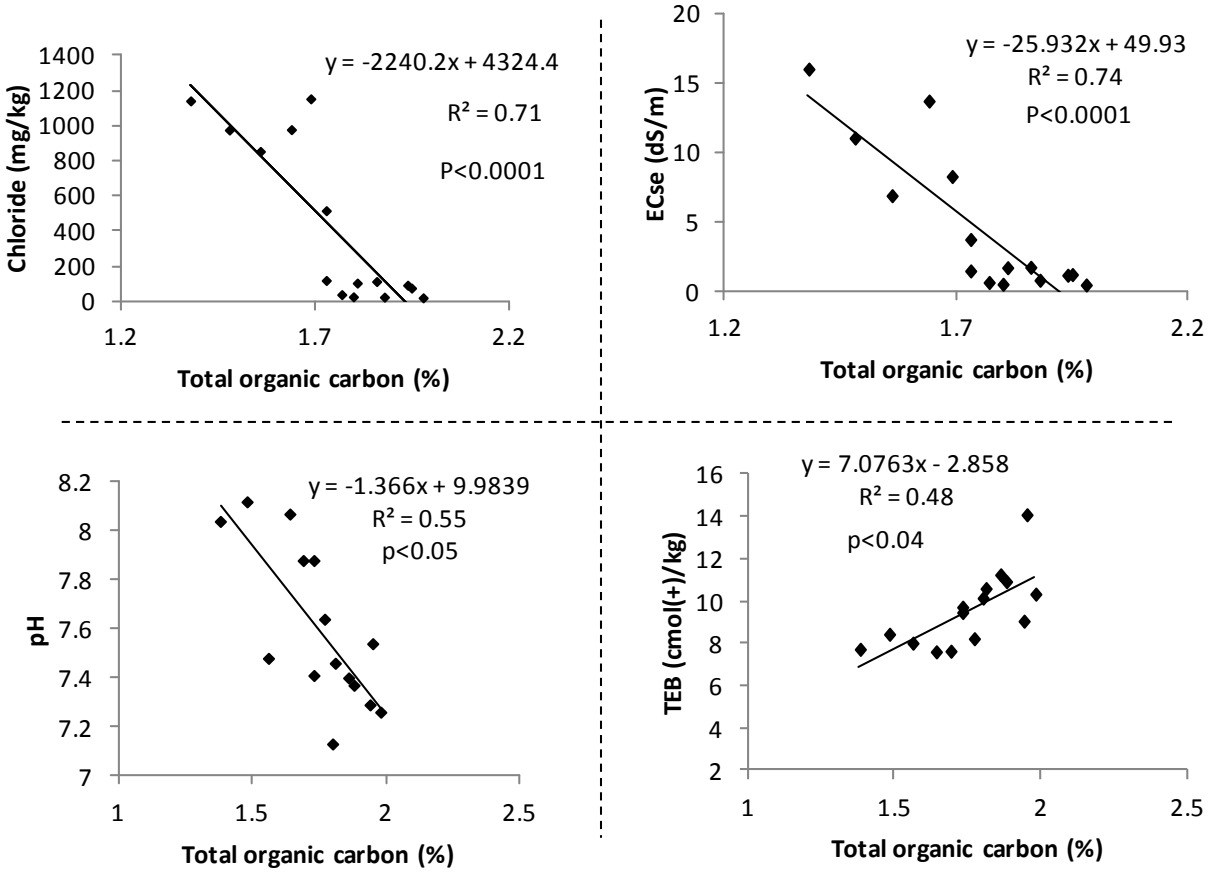

Fig. 16. Correlation between total organic carbon(0-10 cm soil depth only) with other selected chemical properties.

Slope of the regression line between amount of salt applied via saline water and selected soil properties indicates the degree to which saline irrigation affects the soil properties. The greater the magnitude of the slope, the steeper the line and the greater the rate of change in that particular property**.** Table 4 demonstrates that saline irrigation had the greatest influence on ECse and ESP up to a depth of 50 cm. For example, the value of slope (0.0064) indicates that for each kg of salt added per plot (size 105m^2), the ECse value increases, on average, by 0.006 times in 0-10 cm soil depth.

Table 4. Slope of linear relationship between cumulative amounts of salt applied (kg/105 m^2 plot) and selected chemical properties.

Parameters	Soil profile depth (cm)					
	0 –10	10 – 20	20 – 30	30 – 50	50 – 100	100 – 200
ECse	0.0064[a]	0.0040[a]	0.0048[a]	0.0043[a]	0.0030[b]	0.0028[b]
Exch.Ca^{2+}	-0.0024[a]	-0.0031[a]	-0.0034[a]	-0.0032[b]	0.0027[b]	0.0017[c]
Exch.Mg^{2+}	-0.0005[a]	-0.0019[a]	-0.0042[a]	-0.0055[a]	-0.0018[c]	-0.0012[c]
Exch. K^+	-0.00005[c]	-0.0001[a]	-0.00009[a]	-0.0001[a]	-0.00003[c]	-0.00004[c]
Exch.Na^+	0.0014[a]	0.001[a]	0.0007[b]	0.0005[c]	-0.0002[d]	-0.0007[b]
TEB	-0.0015[a]	-0.0041[a]	-0.0071[a]	-0.0084[a]	0.0007[d]	-0.0002[d]
ESP	0.0193[a]	0.0255[a]	0.0258[a]	0.0185[a]	-0.0007[d]	-0.0024[b]

Where, [a] R^2 = >0.80; [b] R^2= 0.60 – 0.80; [c] R^2 = 0.24 – 0.60; and [d] R^2= <0.24 and minus sign indicates negative relationship and positive sign indicate positive relationship. ECse: Electrical conductivity of soil saturate paste extract, Exch.Ca^{2+}: Exchangeable calcium, Exch. Mg^{2+}: Exchangeable magnesium, Exch. Na^+: Exchangeable sodium, Exch. K^+: Exchangeable potassium, TEB: Total exchangeable bases, ESP: Exchangeable sodium percentage.

6.4. Discussion

6.4.1. Soil salinity and sodicity increased with increased concentration of salt in the irrigation water

In this study irrigating with saline water with an EC_w of 16 dS/m over a 2.5-year period caused a non-saline soil (< 2 dS/m) to became highly saline in the topsoil (0 – 10 cm), moderately saline in the lower A horizon and upper B horizon (20 – 50 cm), and highly saline in the subsoil (75 – 150 cm, see Fig. 6). Soils are considered saline if the saturation paste extract ECse is more than 4 dS/m and soil ECse between 4 – 8 and 8 – 16 dS/m are categorised as moderately saline and highly saline, respectively (U.S. Salinity Laboratory Staff, 1954). The reduction in ECse at approximately 20 – 30 cm depth is attributed to lateral subsurface flow through the A2 horizon which is common in these

texture contrast soils (Hardie et al., 2013). Importantly, irrigation of 0.8 dS/m water, equivalent to the upper limits of salinity for domestic wastewater, at 200 and 800 mm/year had no significant effect on soil ECse. The soil ECse values in Plots 7 and 9 (which were irrigated with 16 dS/m saline water at 200 and 800 mm/year irrigation rates) were lower than the salinity of the irrigation water (ECw =16 dS/m), which suggests that capillary rise and surface evaporation of irrigation water played no role in salt accumulation. The higher ECse in 50 – 100 cm soil layer in Plots 7 and 9 indicate that salts accumulated in the lower to mid subsoil clays layers rather than the upper B2 horizon at 30 – 50 cm depth. The apparent conductivity maps also clearly demonstrate accumulation of soluble salts within all plots irrigated with 8 dS/m and 16 dS/m saline water but not in Plots 1 and 3 that received 0.8 dS/m saline water (Fig. 2). Higher apparent conductivity was observed in Plot 9 irrigated with 16 dS/m water at 800 mm/year compared to Plot 3 (0.8 dS/m water at 800 mm/year). This variation is due to the higher amount of salt applied to Plot 9 compared to Plot 3 (Table 3). The EM38 and soil chemical data indicate that irrigation with 0.8 dS/m (wastewater) at 200 and 800 mm/yr for 2.5 years resulted in little increase in root zone soil ECse. Salt accumulation in the soil profile can be a major constraint for crop production due to the reduced ability of plants to uptake water and nutrient from the soil (Dang et al., 2008).

A soil is considered sodic when there is a high proportion of sodium ions relative to other cations present in the soil (Rengasamy et al., 2010) and these can cause adverse effects on soil structural stability and plant growth. In our experiments, irrigation with 16 dS/m water significantly increased sodicity (ESP >15 %) to a depth of 0 – 50 cm, whilst irrigation with 0.8 dS/m water had no significant effect on sodicity throughout the soil profile. Consequently irrigation with 16 dS/m water over a 2.5 year period has resulted in previously non-sodic topsoils becoming highly sodic, whilst previously marginally sodic subsoils (<50 cm depth) have become non-sodic to marginally sodic. Increased topsoil sodicity principally resulted from accumulation of Na^+ ions and reduction of Ca^{2+}, Mg^{2+} and K^+ ions. In Israel, Mantell et al. (1985) also reported that significant increase in ESP to a depth of 0 – 50 cm in loess (Typic Xerothent) after 2 years of irrigation with 7.3 dS/m water under drip irrigation. Strongly sodic soils at or near the soil surface are at considerable risk of structural damage due to induced dispersion of clay aggregates

(Kijne and Kuper, 1995; Agassi et al., 2003). Whilst being frequently irrigated by saline water, the electrolyte content of the soil solution will prevent clay swelling and dispersion, but once irrigation ceases, leaching of soluble salts combined with low electrolyte rainfall is expected to cause soil dispersion in the surface layer leading to surface sealing (Rengasamy and Olsson 1993), loss of porosity, reduced soil moisture holding capacity and infiltration rates (Shainberg and Letey 1984; Patterson, 2006) increased runoff and reduced drainage (Ahuja 1990; Shainberg et al., 1981). These factors are important to the soil type under consideration as it is a strong texture-contrast soil with only weak to moderate topsoil structures and heavy clay subsoils. The possible deterioration of soil physical properties due to saline water irrigation may raise concerns for its long term use in this soil type.

6.4.2. Variation in soil pH between the selected plots after saline water irrigation

This study showed that irrigation with 16 dS/cm water significantly increased soil pH at 0 – 10 cm depth. However, there was a significant reduction in pH (both water and $CaCl_2$) below 50 cm depth (Fig. 5). Increased pH near the soil surface is thought to be due to the increase in exchangeable Na^+ and the displacement of both H^+ and Ca^{2+} ions. In the subsoil, the decreased pH is attributed to clear leaching of Mg^{2+} and K^+ but also the probable addition of H^+ displaced by the Na^+ above 50 cm. Interestingly the greatest reduction in subsoil pH occurred for the two highly saline treatments, rather than the two highest irrigation treatments which supports the notion that leaching and displacement of cations was better facilitated by saline water than purer water. Whilst saline irrigation increased the subsoil concentration of exchangeable Ca^{2+}, the concentration of Mg^{2+}, K^+ and Na^+ all decreased relative to the Control Plot leading to reduced pH at 50 – 200 cm. This is as would be expected from the physio-chemical properties of the ions - i.e., their ratio of charge to hydrated radii. Similar studies have been reported showing such reductions in soil pH in salt treated soils due to the displacement of H^+ by Na^+ ions (Ghallab and Usman, 2007). It has also been suggested that when key base cations are depleted by leaching and replaced by H^+ and Al^{3+} ions thus reducing soil pH (Bates and

Johnston, 1985). However the significant reduction in soil pH in the subsoil in this study showed no associated reduction in TEB cations and suggests that changes in the cation ratios themselves, such as the reduction in ESP is a more likely cause of this significant reduction in pH.

6.4.3. Concentration of exchangeable Ca^{2+}, Mg^{2+} and K^{+} ions decreased in active root zone depth after saline water irrigation

In this experiment we showed a reduction of the base nutrient cations (Ca^{2+}, Mg^{+}, K^{+}) in the upper 50 cm of the profile which is due to their displacement by Na^{+} ions in the saline water. The displacement of these cations is due to ion-exchange processes that follow the lytrophic series and charge of the hydrated ions involved. Irrigation with 16 dS/m water at 800 mm/year leached exchangeable Ca^{2+}, Mg^{2+} and K^{+} in the upper soil profile (0 – 50 cm) to deficient levels whilst Na^{+} increased to toxic levels (Hazelton and Murphy, 2007). Part of the reason for low levels of nutrient cations may be due to the fine sandy loam texture in the upper 20 cm particularly monovalent ions such as K^{+} (Rengasamy et al., 2003). The imbalance of the base cations will affect nutrients in the soil solution and so can have significant impacts on plant growth and function.

Accumulation of exchangeable Na^{+} was more pronounced in the sandy upper layers (0 – 20 cm) than the clay subsoil (50 – 200 cm). Higher exchangeable Na^{+} in the soil surface in saline-water irrigated plots was a result of applied salt via water and evapotranspiration (Burrow et al., 2002). This accumulated exchangeable Na^{+} on soil colloids can affect the survival of vegetation in the long term (Jalali and Merrikhpour, 2008). Na^{+} at high levels outcompetes other nutrient cations for uptake or levels become directly toxic (Rengasamy et al., 2003). High concentration of Na^{+} in the soil solution reduces K^{+} uptake and decreases the Ca^{2+} availability, transport and mobility to growing regions of the plant which ultimately affects the plant growth and yield (Grattan and Grieve, 1999). Higher Na^{+} in irrigation water causes K^{+} desorption and leaching (Bartal et al., 1991; Jalali and Merrikhpour, 2008).

The balance between Ca^{2+} and Mg^{2+} ions in the soil also is considered important for soil structural stability and plant nutrition. A Ca/Mg ratio greater than 6 results in Mg

deficiency, while less than 2 causes Ca deficiency in soils (Hazelton and Murphy 2007). In this study soil irrigated with 16 dS/m saline water caused Mg deficiency in between 0 – 50 cm soil depth (Fig. 14). However, Rengasamy et al. (2003) concluded that deficiencies of Mg or Ca are of much less importance than deficiencies of other major nutrients (N, P and K) on the highly weathered soils of the semi-arid regions.

6.4.4. Significant relationships between total organic carbons (TOC) with selected chemical properties in the topsoil

Total organic carbon (TOC) has a significant role in soil biological, physical and chemical functions (Charman and Roper, 2007). Irrigation with 16 dS/m water significantly lowered TOC at 0 – 10 cm depth. TOC at surface soil (0 – 10 cm depth) was significantly negatively correlated with salinity (ECse), exchangeable Na^+ and Cl^- (Fig. 16). These results agree with similar findings reported in Chinese grasslands (Pan et al., 2013). Reduction in TOC with increased EC is attributed to the reduction of microbial activities and changes in the proportion between fungi and bacteria (Pankhrust et al., 2001). Bacteria/fungi ratios are higher in salt affected soil, as fungi are more sensitive to salinity compared to bacteria (Pankhrust et al., 2001; Sardinha et al., 2003). The reduction of fungal communities may have significant effects on the organic matter decomposition as fungi have a major role in the breakdown of lignin and cellulose of plant residues (Killham, 1994; Crecchio et al., 2004). Other researchers have also described the effect of salt in reduction of soil organic carbon mineralization (Rasul et al., 2006; Wichern et al., 2006; Walpola and Arunakumara, 2010).

We also found a negative relationship between pH and TOC. It has been reported that an increase in pH may result in dissolution of soil organic matter (You et al., 1999). Similar results were also reported in New Zealand, where soil organic carbon was found to be significantly higher when the soil pH decreased in the Pallic Soils (yellow-grey earths) in a 600 mm rainfall zone (McIntosh and Allen, 1993). This significant negative relationship between soil pH and SOC suggests that decomposition of soil organic carbon may have been inhibited at lower pH values as suggested by Shi et al. (2012) and Francis (1986).

6.4.5. Possible plant and soil interactions in saline water irrigated plots

In a salt-affected soil crop performance may be adversely affected by salinity-induced nutritional disorders, which may produce yield penalties (Naidu and Rengasamy, 1993; Grattan and Grieve, 1999). In this study, the accumulated high level of Na^+ and Cl^- on soil irrigated with 16 dS/m saline water (Plot 7 and 9) is likely to affect the survival of non-halophytic plants. High Na^+ levels reduce net K^+ uptake while the higher Cl^- concentration hinders NO_3^- uptake by the plant (Grattan and Grieve, 1999). The high Cl^- concentration (>600 mg/kg) in the soil profile of Plots 7 and 9 indicate a potential for toxic levels of Cl^- (Department of Natural Resources and Water, 2007; Dang et al., 2008). In addition, soil Cl^- concentration is also considered to be a reliable indicator of the ability of roots to extract water (Dang et al., 2008). For a plant growth point of view the high Cl^- may be equally detrimental as high Na^+ in the soil for some species. Recently, both Na^+ and Cl^- were shown to be growth limiting factors in barley (Tavakkoli et al., 2010) despite traditionally detrimental effects of salinity in cereals being attributed to predominantly Na^+ toxicity (Greenway and Munns, 1980). However, the Na^+ and Cl^- accumulation in the plots irrigated with 16 dS/m water are not expected to effect the production of halophytic crops such as species of *Atriplex*. These plants are capable of growing in soil with salinity levels greater than 20 dS/m, or even with sea water irrigation (Glenn et al., 1999; Jordan et al., 2009; Panta et al., 2014) as they are equipped with a salinity tolerance mechanism (Flowers et al., 2010).

6.5. Summary and conclusion

Irrigation water quality significantly influenced the chemical properties of both the sandy loam topsoil and medium clay subsoil horizons of a texture-contrast soil (Brown Sodosol) in a dry subhumid warm climate. Irrigation with 16 dS/m water resulted in significantly higher ECse, Cl^-, and ESP in the upper soil profile. These changes are expected to dramatically impact the growth and yield of almost all crops. Saline irrigation resulted in the variable depth displacement of the base nutrient cations based on their physio-chemical properties ($>K^+ > Mg^{2+} > Ca^{2+}$). The leaching of these ions from the upper profile has induced a nutritional imbalance in the main root zone area, and caused

accumulation of Na^+ and Cl^- to levels potentially toxic to traditional crops. At a soil depth of 10 – 30 cm irrigation with 16 dS/m water reduced Mg^{2+} 13-fold, Ca^{2+} four-fold and K^+ five-fold compared to the control plot. Irrigation with 16 dS/m water increased exchangeable Na^+ approximately five-fold to that of the control. We interpret this as Na^+ displacing of Ca^{2+}, Mg^{2+} and K^+ to greater depths in the soil. This cation displacement reduced the subsoil ESP leading to a beneficial reduction in the subsoil pH.

Irrigation with 16 dS/m water significantly increased both sodicity (ESP) and salinity (EC) in the topsoils (<50 cm). High ESP (>15) in non-saline soils has been linked to declines in soil porosity, permeability and water holding capacity elsewhere. The significant negative correlation between TOC and ECse indicates that high saline irrigation can decrease a key measure of soil health. However irrigation with 0.8 dS/m water which is equivalent to the salt concentration of domestic recycled water used for irrigation in the region, had no significant effect on soil ECse, ESP, TEB and Cl^- at most depths after 2.5 years. Consequently use of 0.8 dS/m water for irrigation appears sustainable at least over the short term, whilst irrigation of 16 dS/m water (the salinity in waste water produced from CSG extraction) at 800 mm/yr, and to a lesser extent 200 mm/yr, resulted in detrimental changes in soil chemical properties that are expected to severely impact the growth and yield of most crops and pastures.

Chapter 7

Productivity and forage quality of *Atriplex lentiformis, Atriplex halimus* and *Medicago arborea* grown in response to irrigation with saline water*

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Abstract

The aim of this study was to quantify differences in the yield and the nutritive value of *Atriplex lentiformis, Atriplex halimus* and *Medicago arborea* as irrigated forage crops in Brown Sodosol. Plants were irrigated with sodium chloride dominated saline water with an electrical conductivity of 0.8, 8 and 16 dS/m at the rate of 200, 500 and 800 mm/year rate. The nutritive value of the three plants was assessed on the basis of acid detergent fibre (ADF), neutral detergent fibre (NDF), dry matter digestibility (DMD), dry organic matter digestibility (DOMD), crude protein (CP), ash content and metabolisable energy (ME), as well as the concentration of selected minerals nutrient such as calcium (Ca), potassium (K), sodium (Na) and phosphorus (P). The dry matter (DM) yield of *Atriplex* species was increased with increasing salinity level but DM yield of *M. arborea* was significantly reduced, 2.7 t/ha/year at 16 dS/m water salinity compared to 8.3 t/ha/year at 0.8 dS/m salinity level. The tissue Ca, K, P and Na content varied between species where *M. arborea* had high Ca (1.6%) but others minerals were higher in *Atriplex* species. In *Atriplex* species Ca content was increased with increasing water salinity but it was reduced in *M. arborea.* Overall, the ash percentage and Na content in plant tissue increased with salinity treatment while this trend was opposite for NDF and K content. The CP of all species ranged from 15.5 to 16.5 % of DM which is comparable to

conventional forage species such as alfalfa. The ME of *Atriplex* species was 8 MJ/kg DM but lower than *M. arborea* (10 MJ/kg DM). *M. arborea* had higher DMD (67 % of DM), DOMD (64 % of DM) and NDF (23 % of DM) content than *Atriplex* species. Overall, the CP, DMD, DOMD and ME values were low at 16 dS/m salinity irrigated at 800 mm/year rate. Based on the observation of selected nutritional parameters, these plants can be used as alternative sources of forage for ruminants during periods of feed shortage. Although they possess all the nutrients that are required for ruminants it may not be suitable to feed as sole source of diet as it has high Na content and may have some non nutritional factors which we have not investigated in this experiment .

Keywords: Soil salinity; Soils sodicity; Soil chemical properties; Exchangeable cations.

7.1. Introduction

Globally, halophytic plants are widely distributed in many arid and saline areas and provide an alternative food source for grazing animals (Swingle et al., 1996, Masters et al., 2007; Panta et al., 2014). Palatable halophytic species are often grazed by sheep, goats, camels and wild animals (Gihad and El Shaer, 1994). Animals such as sheep and goats (Swingle et al., 1996, Ben Salem et al., 2010), camels, and cattle (Khan and Ansari, 2008) perform well on certain halophyte-based feeds. However, the successful use of halophytes as forage for livestock production also depends on their biomass production, nutritive value and voluntary feed intake (Norman et al., 2013).

A number of researchers have explored the possibility of halophytes as livestock feed (Glenn et al., 1992; Riley et al., 1994) and more specifically when grown as irrigated crops (Glenn et al., 1991; Miyamoto et al., 1994; Riley and Abdal, 1993). The yield, nutritional qualities and feeding value of some halophytic plants are comparable to conventional forages (Glenn et al 1999, Masters et al., 2007; Khan et al., 2009) but in some species high content of some minerals e.g NaCl, anti-nutritional compounds and non-protein nitrogen affect their use in animal diets (Benjamin et al., 1992, Glenn et al., 1999; Rogers et al. 2005, El Share, 2010). In addition, many researchers reported that halophytic forage fed animals had lower appetite, growth rate and meat quality compared

to those fed on conventional forages (Gihad and El Shaer, 1994; Glenn et al., 1994; Miyatmoto et al., 1994). In contrast, some researchers described a positive effect of halophytic forage on meat quality (Khan and Ansari, 2008) and no negative effect on animal performance (Al- Shorepy et al., 2010; Khan et al., 2009). However, the general nutritional value varies by species, plant age and growing environment, for instance, irrigation water quality (Swingle et al 1996; Robinson et al., 2004). It may also depend on the combination of both salinity and irrigation regime. If plants are stressed they have a tendency to accumulate various secondary metabolites which may have an important protective (antioxidant) role but detrimental to animal health. So, a balance between commercially-acceptable levels of production and nutritional quality is important for the sustainability of these crops as alternative forages.

Halophytic shrubs, especially from the Chenopodiaceae family, are planted in agricultural areas to provide feed for livestock and rehabilitation of salt affected land (Masters et al., 2010). In addition, the International Center for Biosaline Agriculture (ICBA) has also been conducting several collaborative projects with research organizations in Saudi Arabia, Pakistan and Bangladesh for the development of bio-saline fodder and forage production using *Atriplex* species (*A. halimus*, *A. nummularia* and *A. lentiformis* (ICBA, 2007). These plants are often grown on abandoned land where nothing else grows, and there has been little attempt to identify the effect of growing conditions on the nutritional value of these plants.

Previous studies on *Atriplex* species have provided some information on plant biomass yield (Watson, 1990; Watson and O'Leary, 1993) and the effect of an *Atriplex* base forage diet on milk yield and growth rate in animals (Abu-Zanat and Tabbaa, 2006), feeding quality (Alicata et al., 2002), grazing preference (Norman et al., 2004), mineral content (Watson et al., 1994) and to a lesser extent on the effect of growing conditions on the nutritional quality of the plants (Masters et al., 2010). The aim of this study was to determine the effect of different concentrations of saline water (dominated by NaCl salt), applied under different irrigation regimes on the nutritive value of *Atriplex lentiformis*, *Atriplex halimus* and *Medicago arborea*.

7.2. Materials and Methods

The experimental site was located at the University of Tasmania farm, approximately 12 km N-E of Hobart in the Coal River Valley in Tasmania, Australia. Climatic data recorded at Hobart airport, 7.7 km from the experimental site, showed that the average annual rainfall and evaporation in the region were 469 mm and 1314 mm respectively for the period 1981-2010. For the experimental period of 2012 to 2014 the annual rainfall was 460, 488 mm and 439 mm respectively (Bureau of Meteorology, 2015).

The soil at the study site consisted of an aeolian-derived sandy loam (greyish yellow brown - 10YR4/2) A1 horizon (0-20 cm) and a mottled dispersive, vertic clay to sandy clay B2 subsoil (Holz, 1993; Hardie et al., 2012). This soil is classified as a Brown Sodosol (Isbell, 2002).

The experimental design included three salinity levels (0.8, 8 and 16 dS/m), three irrigation levels (200, 500, and 800 mm/year; in addition to the natural rainfall) and three replicates. The saline water was applied on a weekly basis and water ionic properties are shown in Table 1. Approximately 15 cm tall three-month old glasshouse grown seedlings of each of *Atriplex halimus, Atriplex lentiformis,* and *Medicago arborea* were selected for transplantation in the field in December 2011. Individual species plots were 2.1 x 2.1 m and nine plants of each species were sown at 0.7 m spacing in each plot, equivalent to a planting density of ~20 400 plants per ha.

The first biomass yield was recorded after fourteen months from the time of transplanting and thereafter at twelve month intervals. The plants were cut using a hedge trimmer at a height of approximately 40 cm to encourage regrowth and to avoid harvesting too much woody material. Total fresh yield was measured on site and representative samples were collected for determination of dry matter (DM) yield. Samples were dried in a forced-air draft oven at 60°C for 72 h, and reweighed to determine DM yield, expressed as kilograms of DM per ha.

After three years of saline water irrigation forage samples were cut for the nutritional analysis. Approximately 30 cm long terminal stem sections were selected as a representative part of the plant likely to be grazed by livestock. Nine separate samples from each individual plot of each species were collected. Then the harvested plant

materials were dried in a forced draft oven at 60°C for 72 h. The dried plant materials was ground to <1 mm and sent to FEEDTEST laboratories, Werribee Victoria, Australia for analysis using wet chemistry. Crude protein (Nitrogen x 6.25), neutral detergent fibre (NDF), acid detergent fibre (ADF), dry matter digestibility(DMD), dry organic matter digestibility(DOMD), Ash (%) and minerals (Ca, K, P, Na) were determined, and ME calculated (Mg/kg DM), from the *in-vitro* digestibility (DOMD) using following equation;
ME = (0.203 x DOMD %) - 3.001

Crude protein (CP) was analysed by the Kjeldhl method (Block Digestion), Method 1.4R, page 19. Amylase NDF and ADF were determined by the use of Ankom equipment, Method – 1.8A, page 45 and Methods -1.9 (a), page 60, respectively. While, DMD and DOMD were estimated by pepsin cellulose method, method -1.4R, page 31 as described in laboratory methods manual published by Australian Fodder Industry Association Ltd (2011). A dried, ground sample was ignited in a furnace at 600˚C to oxidise all organic matter and ash is determined by weighing the resulting inorganic residue.

The minerals content (Na, K, P and Ca) of sample was determined by a Varian Vista-MPX simultaneous ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) with CCD detection (Ryan, 2005).

Table. 1. Composition of irrigation water used for the experiment (waters samples were analysed in CSBP laboratory in Western Australia).

Water treatment	pH	Conductivity dS/m	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	P mg/L	SAR
0.8	7.0	0.8	86.8	14.9	22.4	17.3	4.3	3.1
8	7.1	8.0	1567.0	15.0	22.0	16.1	4.1	60.8
16	7.0	16.0	3622.0	16.7	22.3	15.8	4.0	142.4

Statistical analysis

Data were analysed using analysis of variance (ANOVA) to determine if significant differences were present among means for salinity levels, irrigation levels, plant types and interaction between these three factors. To obtain a multivariate view of all variables, the means of all variables were standardised by variable, and subjected to principal component analysis (PCA) (Minitab 15; Minitab Inc.).

7.3. Results

An ANOVA showed an individual effect of salinity level, irrigation rate and plant species, and an interaction between salinity level and irrigation rate, salinity and species, and irrigation rate and species for a number of the parameters measured (Table 2). There was a significant individual effect of species for all measured parameters. The salinity levels had a significant effect only on the ash and Na^+ content of the forage.

Table 2. The significance probability associated with the F statistic (Pr>F value) of three- way ANOVA of salt level (S), irrigation rate (IR) and species (SP) treatments.

Sources	P value											
	Yield	ADF	NDF	DMD	DOMD	Ash	CP	ME	Ca	K	Na	P
S	0.200	0.472	0.007	0.288	0.292	0.017	0.136	0.328	0.112	0.050	0.018	0.112
IR	0.251	0.596	0.237	0.766	0.774	0.823	0.238	0.776	0.495	0.228	0.291	0.105
S*IR	0.594	0.182	0.119	0.024	0.022	0.104	0.019	0.016	0.717	0.268	0.097	0.788
SP	<0.000	<0.00	<0.000	<0.00	<0.000	<0.00	0.026	<0.00	<0.00	<0.00	<0.00	<0.00
S*SP	<0.000	0.207	0.098	0.650	0.645	0.000	0.547	0.657	<0.00	0.442	0.202	0.677
IR*SP	0.059	0.097	0.062	0.157	0.161	0.137	0.195	0.029	0.002	0.723	0.775	0.763

Abbreviations: S, salt levels; IR, irrigation levels, SP, species; Yield, dry biomass yield; ADF, acid detergent fibre; NDF, neutral detergent fibre; DMD, dry matter digestibility; DOMD, dry organic matter digestibility; ME, metabolisable energy; Ca, calcium; Yield, biomass yield; P, potassium; Na, sodium; K, potassium; CP, crude protein.

There was no difference ($p>0.05$) in the yield of plants grown under different irrigation regimes. However, there was a significant interaction between salt level and species ($p<0.0003$). There was a general trend of increasing biomass accumulation for *A. lentiformis* and *A. halimus* with increasing salinity levels in irrigation water, while, in *M. arborea* the trend was opposite. At 16 dS/m water salinity *A. halimus* had the highest dry matter (DM) yield of 24.6 t/ha/year followed by *A. lentiformis* (16.8 t/ha) while *M arborea* yield was significantly less (2.8 t/ha/year) (Fig.1).

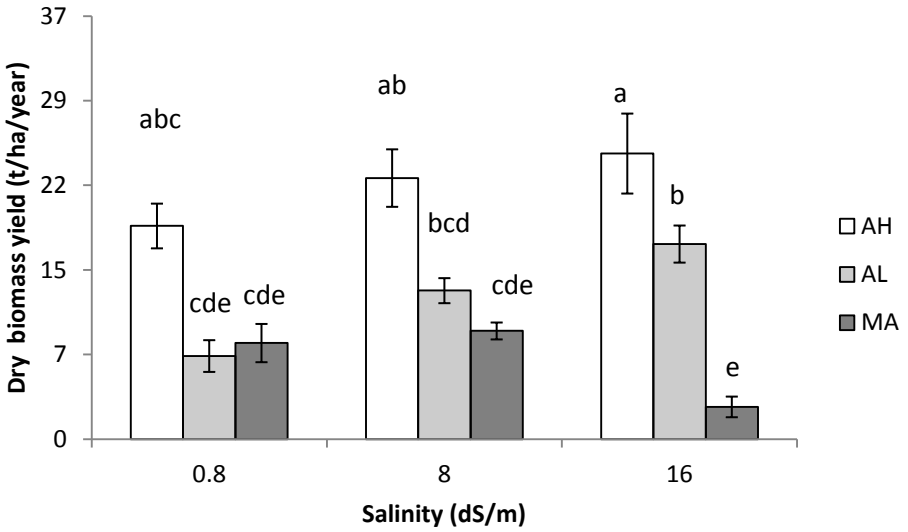

Fig.1. The mean dry biomass yield (t/ha/year) of *Medicago arborea*, *Atriplex lentiformis* and *Atriplex halimus* in response to three irrigation water salinity levels 0.8, 8 and 16 dS/m. Vertical bars represent standard errors. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The simulated option mean difference was 7.3.

The selected forage quality parameters measured in this experiment significantly differed among species ($p<0.01$; Table 2). However, the individual effect of salinity and irrigation rate had different effects depending on the parameters tested. Similarly, there was a significant difference ($p<0.01$) between species for ADF and NDF (Table 2). The

highest ADF (Fig. 2) and NDF (Fig. 3) values were obtained from *M. arborea* compared to the *Atriplex* species.

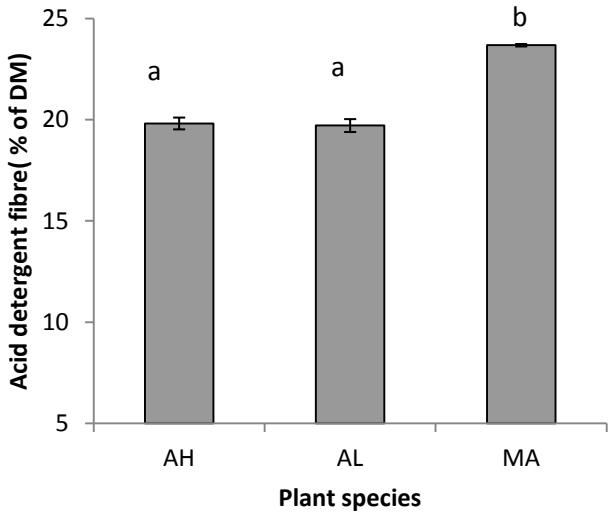

Fig. 2. The mean acid detergent fibre (ADF) content of *Atriplex halimus* (AH), *Atriplex lentiformis* (AL), and *Medicago arborea* (MA) in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500, and 800 mm per year. Vertical bars represent standard errors. Different letters above bars represent significant ($p<0.01$) difference among treatments where mean difference was based on Simulated Option in SAS. The simulated option mean difference was 0.94.

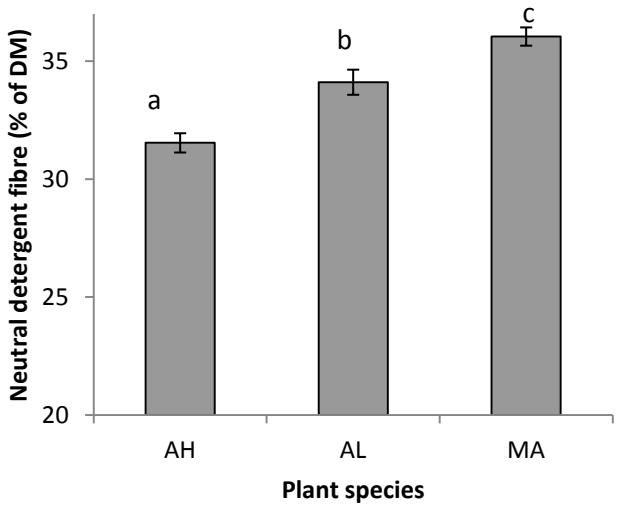

Fig. 3. The mean neutral detergent fibre (NDF) content of *Atriplex halimus* (AH), *Atriplex lentiformis* (AL), *Medicago arborea* (MA) in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500, and 800 mm per. Vertical bars represent standard errors. Different letters above bars represent significant ($p<0.01$) difference among treatments where mean difference was based on Simulated Option in SAS. The Simulated Option mean difference was 1.42.

The DMD, DOMD, CP and estimated ME were not influenced by the salinity level of the applied water, however there was a significant interaction between salt levels and irrigation rates on these nutritive descriptors ($p<0.05$; Table 2). Fig. 4 and 5 shows that DMD and DOMD were lower in 16 dS/m salinity treatment when applied with 800 mm of irrigation/year. Similarly, CP and ME were also lower for this treatment combination (Fig. 6 and 7). A comparison of species showed that *M arborea* had significantly higher DMD and DOMD compared to *A. halimus* and *A. lentiformis* **(**Table 3). Slightly higher CP (16.4 % of DM) and ME (10.0 MJ/kg of DM) were found in *A. halimus* and M. *arborea* respectively.

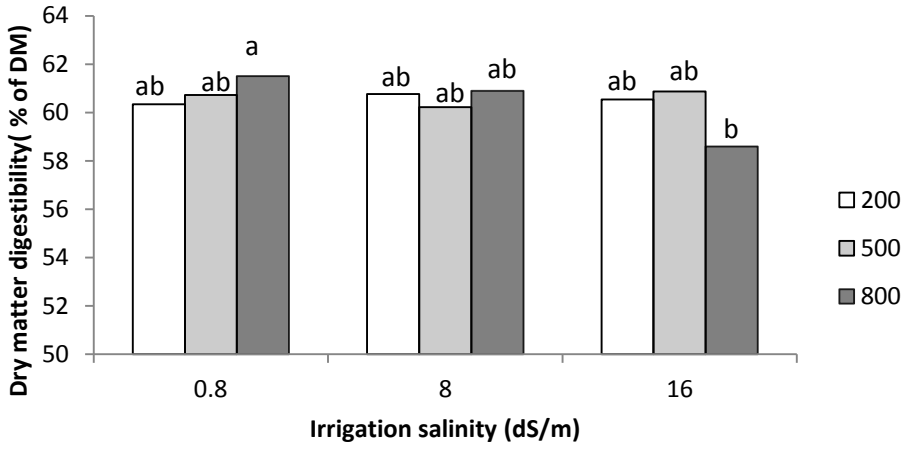

Fig. 4. The mean dry matter digestibility (DMD) (% of DM) of plants (values are average of *Medicago arborea*, *Atriplex lentiformis* and *Atriplex halimus* plant species) in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500 and 800 mm per year. Different letters above bars represent significant ($p<0.05$)

difference among treatments where mean difference was based on Simulated Option in SAS. The simulated option mean difference was 2.89.

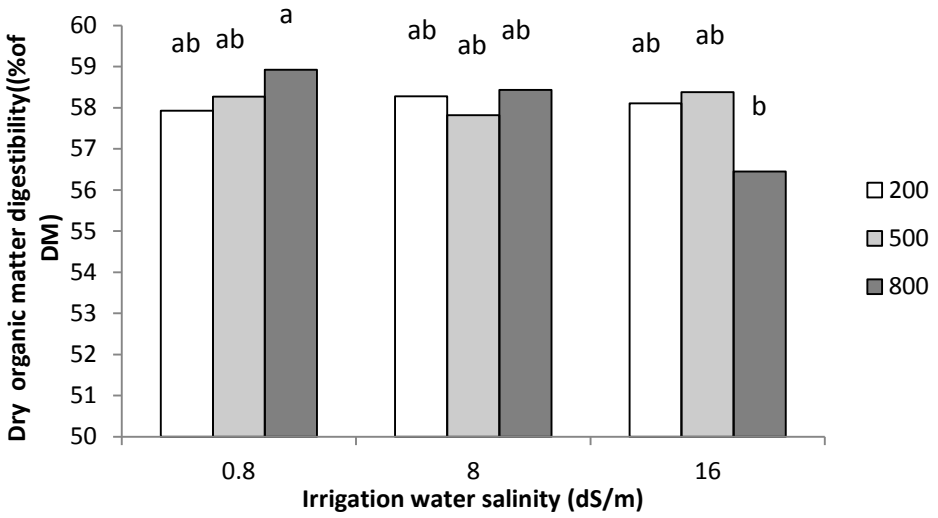

Fig. 5. The mean dry organic matter digestibility (DOMD) (% of DM) of plants (values are average of *Medicago arborea*, *Atriplex lentiformis* and *Atriplex halimus* plant species) in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500 and 800 mm per year. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The Simulated Option mean difference was 2.35.

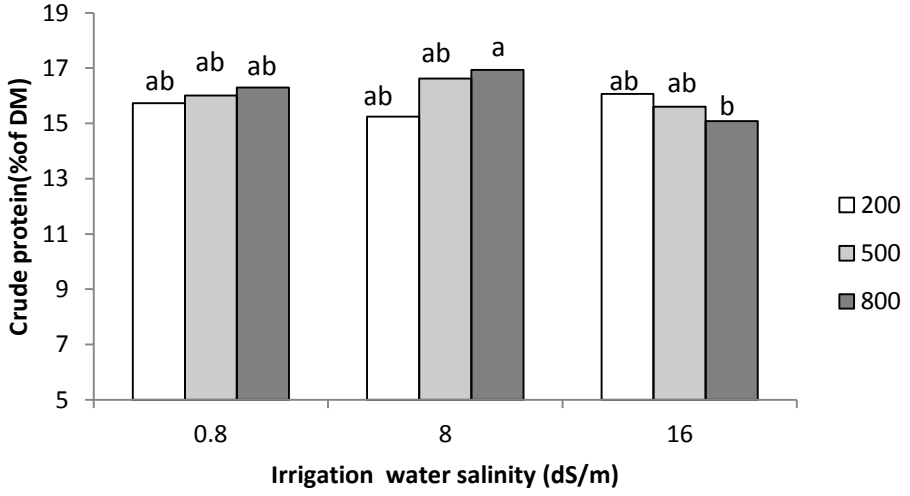

Fig. 6. The mean crude protein (% of DM) of plants (values are average of *Medicago arborea*, *Atriplex lentiformis* and *Atriplex halimus* plant species) in response to three

irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500 and 800 mm per year. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The Simulated Option mean difference was 1.73.

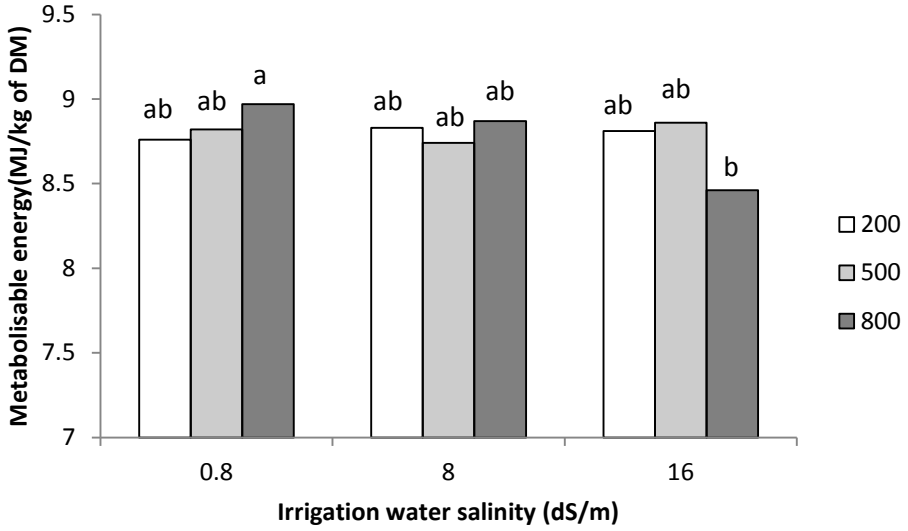

Fig. 7. The mean metabolisable energy (mg/kg) of plants (values are average of *Medicago arborea*, *Atriplex lentiformis* and *Atriplex halimus* plant species) in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500 and 800 mm per year. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The Simulated Option mean difference was 0.48.

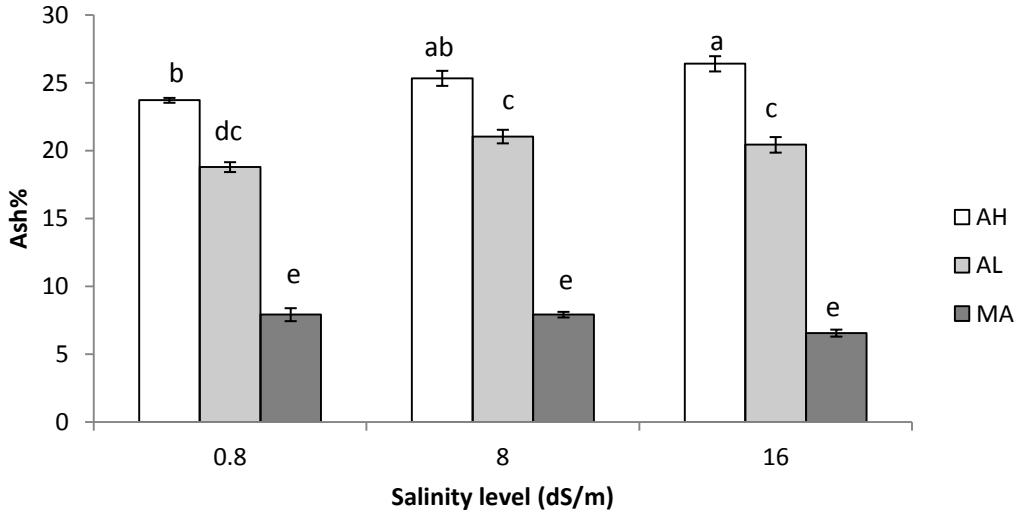

Fig. 8. The mean ash content (%) of *Atriplex halimus* (AH), *Atriplex lentiformis* (AL) and *Medicago arborea* (MA) forage in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m. Vertical bars represent standard errors. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The simulated option mean difference was 1.91.

The ash content of the forage species was affected by the salinity level ($P< 0.05$) and there was a significant interaction between salinity level and species (Fig. 8). *Atriplex halimus* had the highest ash content at all salinity levels, compared to *A. lentiformis* and *M. arborea*. In *A. halimus* there was a significantly higher ($p<0.05$) ash content at 16 dS/m compared to the 0.8 dS/m treatment. A comparison between species showed that *A. halimus* had the highest ash content (26.4% of DM) while *M. arborea* had the lowest content (6.5 % of DM) at 16 dS/m irrigation salinity (Fig. 8).

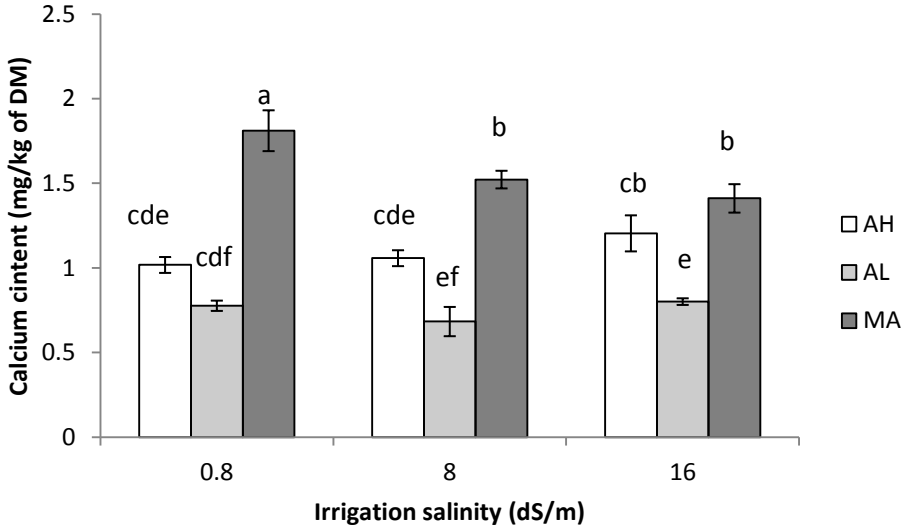

Fig. 9. The mean calcium content (mg/kg of DM) of *Medicago arborea* (MA), *Atriplex lentiformis*(AL) and *Atriplex halimus* (AH) forge in response to three irrigation water salinity levels; 0.8, 8 and 16 dS/m and irrigation rates of 200, 500 and 800 mm per year. Vertical bars represent standard errors. Different letters above bars represent significant ($p<0.05$) difference among treatments where mean difference was based on Simulated Option in SAS. The Simulated Option mean difference was 0.285.

The selected minerals content (Ca, K, P and Na) in the forage was significantly different between the species ($p<0.0001$) (Table 2). In addition, there was a significant interaction between species and salinity level for Ca content in the forage (Fig. 9) The mean values show that forage K and P were significantly higher ($p<0.0001$) in *A. lentiformis* and *A. halimus* compared to *M. arborea* (Table 3). On the other hand, forage Na content was significantly influenced by salt levels and values were varied between plant species but there was no interaction between them. The highest forage Na content was recorded from the 16 dS/m saline water treated plants. *A. halimus* (6.99 % of DM) had highest Na content followed by *A. lentiformis* (4.57 % of DM) and *M. arborea* (0.25 % of DM) (Table 3 and 4).

Table. 3. Least square means of plant species effect on measured characteristic.

Species	Least Squares Means								
	DMD	DOMD	Ash	CP	ME	Ca	K	Na	P
	(% of DM)	(% of DM)	(% of DM)	(% of DM)	MJ/kg DM	(% of DM)	(% of DM)	(% of DM)	(% of DM)
AH	57.47 a	55.50 a	25.14a	16.42a	8.26 a	1.09a	1.68a	6.99a	0.26a
AL	56.51a	54.69 a	20.08b	15.51 b	8.11 a	0.75 b	1.96 b	4.57 b	0.22b
MA	67.50 b	64.00 b	7.46 c	15.95 ab	9.99 b	1.58c	1.58c	0.25 c	0.16c
(MSD)	1.23	1.06	0.82	0.77	0.21	0.08	0.20	0.50	0.02

Values in each column with the same letter are not significantly different at $p<0.05$. Where, AH, *Atriplex halimus*; AL, *Atriplex lentiformis*; MA, *Medicago arborea*; MSD, Simulated Option Mean Significance Difference, DMD; Dry Matter Digestibility; DOMD, Dry Organic Matter Digestibility; CP, Crude Protein; K, Potassium; Na, Sodium; P, Phosphorus content and ME; Metabolisable Energy.

Table. 4 Least square means of salt level effect on NDF, Ash, K and Na. Unit are % of DM.

Salt level (dS/m)	Least Squares Means			
	NDF	Ash	K	Na
0.8	34.89 a	16.81a	1.76 a	3.72a
8	33.66 ab	18.10 b	1.54 bc	3.71a
16	33.14 b	17.80 b	1.49c	4.35b
Mean significance difference (MSD)	1.41	0.82	0.19	0.48

Values in each column with the same letter are not significantly different at $p<0.05$.

Abbreviations: NDF, Neutral Detergent Fibre; K, Potassium; Na, Sodium content.

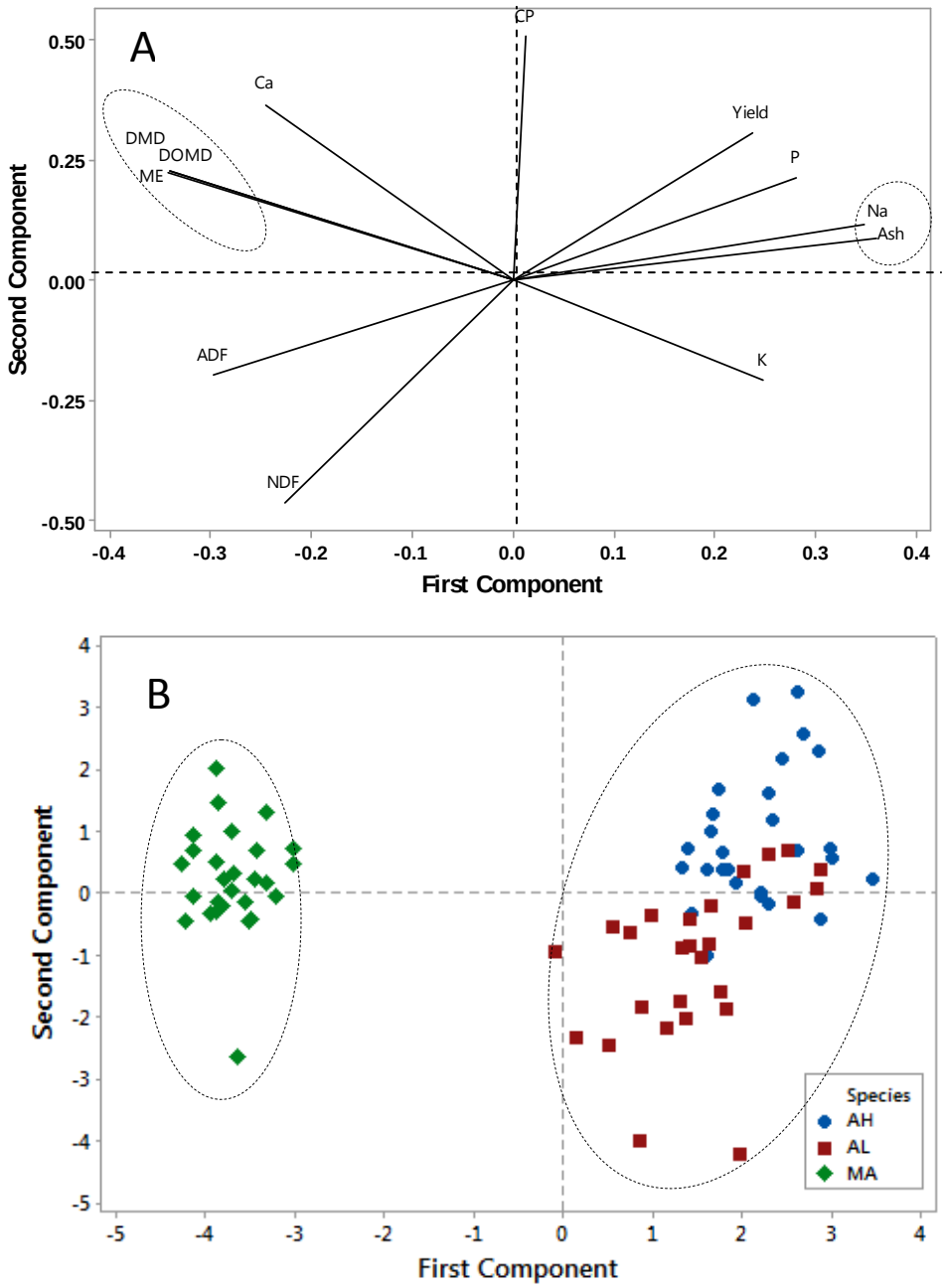

Fig. 10. Plots of principal component 1 *versus* principal component 2 based on measured characteristics of three plant species. The loading plot shows the eigenvector of each characteristic. The score plot shows the measured characteristic grouped by plant species. Abbreviations: ADF, acid detergent fibre; NDF, neutral detergent fibre; DMD, dry matter digestibility; DOMD, dry organic matter digestibility; ME, metabolisable energy; Ca, calcium; Yield, biomass yield ; P, potassium; Na, sodium; K, potassium, AH; *Atriplex halimus*; AL, *Atriplex lentiformis*; MA, *Medicago arborea*.

Relationships of parameters measured under saline conditions were obtained using principal component analysis. Figure 10 shows the relationship between the parameters measured under saline conditions (Fig. 10A) and species used in this study (Fig. 10B). In Fig. 10A, the cosine of the angle between two characteristics estimates the correlation between them. Thus, lines that were clustered were positively correlated, lines at 90° to each other were uncorrelated, and lines with the angle close to 180° were negatively related. The major nutritional characteristics measured (metabolisable energy (ME), dry matter digestibility (DMD) and dry organic matter digestibility (DOMD) appeared in the same quadrant (Fig. 10A) while sodium (Na) and ash content were in another quadrant with a positive relationship. This suggests shoot Na content could be an important determinant of forage ash content under saline irrigation. No clear association between NDF, ADF and yield (or any other nutritional characteristics) was found (Fig. 10A). The location of sample point (species) on the principal components plot also contributed to the understanding of relationship between species and measured parameters (Fig. 10B). A clear grouping between *M. arborea* and *Atriplex* species were observed (Fig. 10B). Further insights into the relationship of measured parameters in individual species were shown in Fig.11. Ash content in the *M. arborea* was influenced by Ca and K content but in *Atriplex* species accumulated Na ion was the main contributor for ash content.

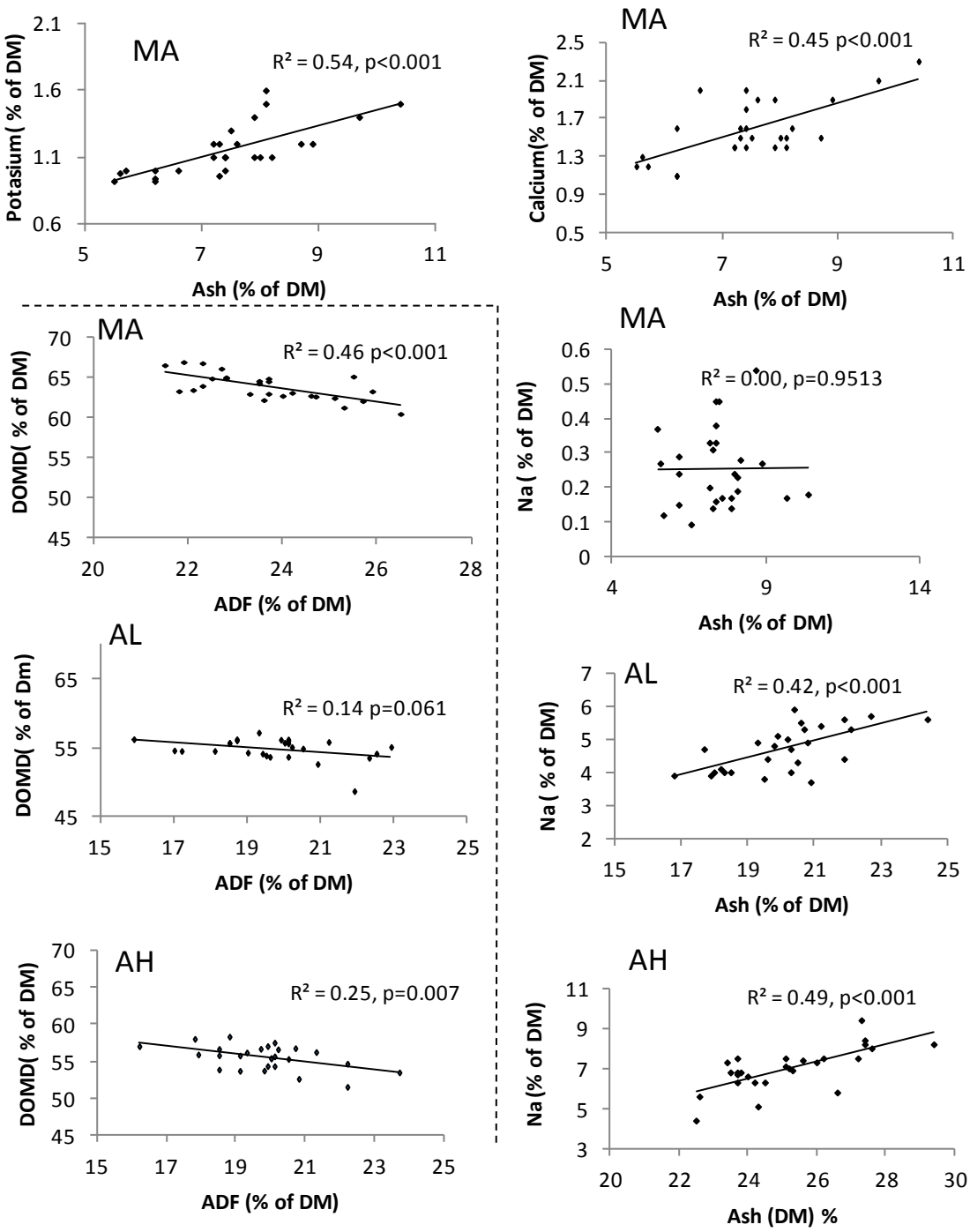

Fig. 11. Correlation of selected parameter of individual species grown in saline environment. Where MA; *Medicago arborea*, AL; *Atriplex lentiformis*; AH; *Atriplex halimus.*

7.4. Discussion

7.4.1. Salinity caused variation in dry matter yield between plant species

In this study biomass yield varied considerably between plant species. *A. halimus* had the highest yield followed by A. *lentiformis* and *M arborea*. The *M arborea* yield was higher at 8 dS/m salinity (DM 10 t/ha), however as salinity increased to 16 dS/m, its biomass was significantly reduced (DM 2.78 t/ha) (Fig. 1). The *Atriplex* species were seen to benefit by the increased salinity level in irrigation water with higher dry matter yield (>20 t/ha) at 16 dS/m water salinity). This suggests that physiological activity in *M. arborea* is adversely affected beyond the 8 dS/m water but that was not observed in *Atriplex* species up to 16 dS/m. The reduction of *M. arborea* growth at 100 mM NaCl (~10 dS/m) (Sibole et al. 2003; Boughalleb et al. 2009a,b) and optimal growth of *Atriplex* species were observed at 200 mM NaCl salinity (~20 dS/m) (Greenway and Munns, 1980; Flowers and Colmer, 2008). Salinity can cause deleterious effects on plants which are thought to be a result of ion toxicities and ion imbalance, or a combination of both (Ungar, 1991). But halophytic plants which have a capacity to adjust tissue ions and even benefit within a certain range of salinity levels (Flowers et al., 1977; Flowers and Colmer, 2008,). In saline soil, plants can also suffer from a deficiency of certain nutrients. Saline water can cause high concentrations of Na^+ and reduced amounts of K^+, Mg^{2+} and Ca^{2+} in the topsoil or active root zone area in the soil profile (Epstein, 1972) and thus shallow rooted plants will be affected if grown in such situations.

Also, plant biomass yield can be affected by several environmental and agronomical factors such as waterlogging (Bennett et al., 2009), fertiliser application (Barrett-Lennard et al., 2003), cutting frequency (Bustan et al., 2005) and plant density. A large difference in forage productivity in halophyte species grown in non-irrigated and irrigated systems has been reported. For example, in southern Australia, annual leaf yields of *Atriplex* species under non-irrigated conditions were 0.4 – 0.7 t DM/ha (Malcolm et al., 1988; Norman et al., 2008) but Watson et al., (1987) showed leaf yields of 10 – 20 t DM/ha when irrigated with water of 10 dS/m salinity. It is also notable that at 16 dS/m salinity *M. arborea* could have suffered from both salinity and waterlogging when irrigation rate increased from 200 to 800 mm/year. Thus this species may not grow

well in waterlogged conditions and it is known to be well adapted to drought conditions (Sibole et al., 2003; Boughalleb et al., 2011). But there was no indication of negative effects of either salinity of waterlogging in the *Atriplex* species. This suggests that *Atriplex* species can be successfully grown at high irrigation rates (800 mm/year) and high salinity levels (16 dS/m) in a 600 mm/year annual rainfall zone in a texture-contrast soil.

7.4.2. Forage NDF value was influenced by both plant species and salinity treatments

The results show that nutritive values varied based on the plant species. The NDF values were influenced by both salinity and species. NDF was reduced with increasing salinity levels (Table 4). Robinson et al. (2004) also reported that reduction of NDF by 3% in *Thinopyrum ponticum* (Tall wheat grass*)* that was irrigated with different water salinities (15 or 25 dS/m) but Pasternak et al. (1993) found no such consistent relationship in halophytic grasses grown in soil with different levels of irrigation salinity (1.2 - 9.5 dS/m). The stage of plant maturity is one of the major factors influencing forage quality (Robinson et al., 2004). It was also reported that NDF was increased from 18.6 to 42.6 % from early to late harvest in alfalfa (Stefanon et al., 1996). As ADF and NDF are a measure of fibre content in the plant, its values increase when plant maturity increases (McDonald et al. 2002). The optimal growth of halophytes is associated with succulence of leaves and stems (Bell and O'Leary, 2003; Pardia and Das, 2005). Similarly, increased leaf succulence was found in *Suaeda fruticosa* at 200 mM NaCl salinity (Khan et al 2000b). In addition, in this experiment harvested forage samples were from the top 30 cm of vegetative growth. This reflects that harvested plant parts were actively growing and succulent at the time of harvest and hence had lower NDF values. Nonetheless, these values are comparable to those of traditional forage such as grass and legumes (Fulkerson et al., 2007; Homolka et al., 2012).

7.4.3. Variation in forage ash and ion content based on applied treatment

The higher ash content of *Atriplex* species (18 - 26.4% of DM) in comparison to *M. arborea* (6.5 - 8% of DM) indicates that *Atriplex* species is an ion accumulator (Fig.8). Interestingly these high ash concentrations were correlated with the tissue Na content in *Atriplex* species but not in *M. arborea* (Fig. 11). In general, Chenopods (e.g. *Atriplex* species) contain higher ash (% of DM) than other halophytic grasses or legumes (SCA, 2007). In this study, the *Atriplex* species (halophytes) had higher foliage Na and K content compared to *M. arborea* (salt tolerant glycophyte). Halophytic plants accumulate more Na for osmotic adjustment as cheap osmoticum (Belkheiri and Mulas, 2013; Shabala, 2013). If comparisons were made between plant species *A. halimus* had significantly higher Na, by nearly 1.5 times, than A. *lentiformis* and 28 times higher than *M. arborea*. It is also known that uptake of Na and Cl, and their compartmentalisation in vacuoles is one of the salinity tolerance mechanisms in chenopods (Munns and Tester, 2008; Flowers and Colmer, 2008). K content was higher in *A. lentiformis* by only 1.2 times that of *A. halimus* and 1.3 times of *M. arborea*. It shows that there is less variation in K content between the plant species. This is be supported by the fact that K is a principal electrolyte for plants as shown by their strong preference for K uptake over Na even though there is abundant Na in the external environment (Walker et al., 1996). This kind of discrimination in favour of K uptake shows that a trait has evolved in plants to conserve K in saline environments (Subbarao et al., 2003). Both Na and K are involved in the osmotic adjustment in the plant tissue (e.g. *Atriplex* species) to lower the external water potential in saline conditions (Osmond et al., 1980; Khan et al. 2000a; Flowers et al., 2015).

There was a significant impact of irrigation water salinity on forage ions concentration. Overall, a significantly higher forage Na and significantly lower forage K was observed in plants treated with 16 dS/m irrigation salinity (Table 4). In saline environments halophytic plants accumulate inorganic ions (Na and Cl) to adjust the osmotic potential of their internal tissues to the external salinity (Colmers and Flowers 2008; Flowers et al., 2015). In contrast, low concentration of tissue K found in high salinity treated plants can be explained by the antagonistic relationship between Na and K

uptakes in the plant system (Jeschke, 1984; Bernstein et al, 1995; Hu and Schmidhalter, 2005). In addition to this, under saline environments Na^+ uptake caused strong membrane depolarization and then it favours K^+ efflux through depolarization-activated outward-rectifying K^+ channels in plant cells (Shabala et al., 2006).

It is also notable that increased salinity in irrigation water significantly ($p<0.5$) reduced the tissue Ca^{2+} content in *M. arborea* but this trend was not apparent in the *Atriplex* species (Fig. 9). This suggests that the low tissue Ca^{2+} content in the *M arborea* could be due to the reduction in Ca^{2+} uptake by the plant from the soil solution. This is supported by the soil analysis report showing (see chapter 6) Ca^{2+} concentration in the top 50 cm of the soil profile in the 16 dS/m salinity treatment was very low and was leached down the profile to the subsoil (below 50 cm). This reduced Ca^{2+} availability for plant uptake. The variation in the salinity effect on plant species can also be explained by the structure and biology of the plant. In this case, the plant root system may have played an important role in nutrient uptake. It has been reported that *A. halimus* can often penetrate to a depth of 10 m (Le Houérou, 1980). This extensive root system may enable these species to reach subsoils for Ca^{2+} uptake. On the other hand, *M. arborea* was not growing very well under high salinity treatments (Fig. 12) and has more superficial root system compared to *Atriplex* species. Thus *Medicago arborea* may be unable to take up Ca^{2+} from the subsoil. This notion is supported by the low tissue Ca^{2+} content of *M. arborea* that was grown at high salinity conditions.

Fig. 12. Performance of *Medicago arborea*, *Atriplex halimus* and *Atriplex lentirormis* at16 dS/m salinity treatments.

7.4.4. Forage nutritive values (ME, digestibility and CP) influenced by the plant species but not by applied salt levels

In this study, the ME value of *Atriplex s*pecies was lower than *M. arborea*, which may be due to the lower DOMD values found in *Atriplex* species (Table 3). The amount of DOMD for *Atriplex* species ranged from only 55 to 56% while the salt tolerant glycophyte (*M. arborea*) had a DOMD of 64%. In addition, increased salinity and irrigation levels seem to have reduced the DOMD values in the plant species (Fig. 5). Similarly, estimated ME values changed very little (significant, $p<0.05$) with increasing salinity treatment from 8.97 to 8.8 MJ/Kg DM at higher irrigation rate (Fig. 7). A similar result was reported in *Melilotus speciosus,* where ME declined from 10.5 MJ/kg DM at 0 mM NaCl to 8.8 MJ/kg DM at 240 mM NaCl (Rogers et al., 2008).

The mean CP content for all species ranged from 15.5 to 16.4%, and there was no main effect of the salinity treatments in the CP content which is in agreement with the finding in grass species reported by Pasternak et al. (1993). It is been reported that alterations in the ionic equilibrium in the cells may cause blockage of protein metabolism (Neumann, 1997; Uddin et al., 2012). The data from this study suggests that crude protein was not totally compromised by the salinity levels used. However, plant grown in 16 dS/m water salinity irrigated at high rate (800 mm/year) showed significantly ($p<0.05$) low CP content. The CP content of around 16% is comparable with lucerne hay, and therefore, can be considered of good quality as animal feed. A top quality lucerne hay should have at least 19% CP, 9 MJ/Kg DM ME and 65% digestibility (Lattimore, 2008). These values are considerably higher than the CP content of alternative salt tolerant grass species, *Sporobolus virginicus* (8.7% of DM) and *Distichilis spicata* (9.8 % DM) reported by Al-Shorepy et al. (2010).

Meaning of selected minerals values of the studied plants in ruminant diet

Livestock need an adequate amount of minerals in their diet from available feeds and forages for healthy growth and weight maintenance. However, their requirement varies based on type of animal and their growth condition, for example, a higher plane of nutrition is needed for growing and lactating animals compared to dry or fully grown animals. In ruminants, Na and K have an electrochemical function and are associated

with osmotic control of water in the body, while Ca and P are essential for bone and energy metabolism (McDonald et al., 2002).

In this experiment phosphorus (P) content of the studied plants species was 1.6 to 2.6 g/kg DM (or 0.16 to 0.26 % of DM) which is within the recommended range for ruminants. P deficiency causes loss of appetite and skeletal abnormalities in animals (Suttle, 2010). The recommendation in Australia and New Zealand for P in animal feed is 1.3 – 2.5 g/kg DM) while in the USA and UK it between 1.2 and 3.8 g/Kg DM (Suttle, 2010). In comparison, P content in lucernes and clover silage are 3.3 and 2.3 g/Kg DM respectively (MAFF, 1990). Data from the present study indicates that these plants are comparable with the conventional feed and pose less risk of P deficiency in both growing and mature animals.

In grazing ruminants, Ca requirements are highly dependent on the animal types, age, and body weight and suggested maximum level of calcium in the diet is 1.5% of DM (NRC, 2005). However, the recommended minimum requirement is a subject of debate. In this study the concentration of Ca in *M. arborea and Atriplex* species was 1.5 % of DM (15 g/kg DM) and 0.75 – 1.0 % of DM (7.5 to 10 g/kg DM), respectively. These values are considered adequate for ruminants. For comparison, Ca content in leguminous forages is >10.1 g/kg DM while grasses contain >3.7 g/kg DM (Minson, 1990). In alfalfa silage Ca is reported from 13.5 to 24.0 g/kg DM (MAFF, 1990). It has been also described that diets containing 3 g Ca/ kg DM would not affect the sheep performance. However, this value is varied between countries. For example in Australia, the recommended Ca content for sheep is 1.5 – 2.6 while in USA it is 2.0 – 8.2 g/kg DM (Suttle, 2010). Thus, Ca deficiency in animals would not be expected if fed with these forage species. However, phosphorus content in *M. arborea* was lower than *Atriplex* species and the Ca : P ratio in *M arborea* was 9.8 followed by 4.19 and 3.4 in *A. halimus* and *A. lentiformis* respectively. As Ca and P are interrelated and they must also be supplied in the right proportions and a ratio of Ca: P of more than 3.6 is considered detrimental for sheep (NRC, 2005). These values were more than or close to the maximum tolerable limit for the sheep diet so sheep health may be affected if given as sole feed.

The Na requirement for ruminants is often debated in the literature (Mirzaei, 2012). An adequate range from 1- 4 g/kg has been recommended by various researchers (Underwood, 1981) while in a field trial grazing lambs and ewes showed the minimum Na requirement of 0.5-1.0 g/kg DM (Edmeades and O'Connor, 2003). The data of this study showed that, with the exception of *M. arborea* (2.5 g/kg DM) both *Atriplex* species had very high Na^+ content (45 – 70 g/kg DM). So in animal diets mixing of *Atriplex* species with other low Na content forage species is necessary to avoid Na toxicity. In general, Na content in conventional forages has been reported as less than 1.5 g/ kg DM (Suttle, 2010). However, grasses (e.g. Phalaris species) or legumes (e.g. white clover) commonly contain 3 – 4 g Na kg/DM while *Pennisetum clandestinum* (kikuyu), lucerne and red clover, commonly contain <0.5 g/ kg DM (Edmeades and O'Connor, 2003).

In this study the levels of K in *Atriplex* species were between 17 to 20 g/kg and in *M. arborea* it was 15 g/kg which is above 8 g/kg recommended for grazing animals (Underwood, 1981). The K concentrations in commonly used forages are reported somewhere between 10 to 45 g/kg DM. For example, K content in lucerne hay was 27.5 g/kg (McNeill et al., 2002; Suttle, 2010). It is also reported that the maximum tolerable level of K for sheep is 2 % of the diet DM (NRC, 2005). This indicates that our plant species showed K content below the maximum tolerable level. So problems of K deficiency or toxicity are unlikely to arise if these plant species are used as animal feeds. It was also noted that suboptimal levels of K can result in decreased feed intake and decreased live weight gain (Minson, 1990). Growth rates have been seen to decline in animals when high K content (60 g/kg DM) forage was fed (Scuttle, 2010). Excess K in the diet also causes hyperkalaemia and cardiac arrest in animals (Neathery et al., 1979). In general, the grazing livestock consuming these shrubs would receive adequate to excessive K but would not have deficiency problems.

Although *Atriplex* species had adequate Na and K and meet the requirements for ruminants, sometimes mineral interactions within plants and animals make these plants unsuitable as a sole source of feed. Mayberry et al. (2010) have shown mineral imbalance in sheep when *A. nummularia* was given as sole source of feed. It is also reported that poor animal performance was associated with low digestibility of *Atriplex* species (Norman et al., 2008). In addition, high concentrations of sodium or potassium chloride

in feed or water have been reported to be associated with lower food intake by the animal (Wilson, 1966; Masters et al., 2005). Thus A*triplex* species could serve as an alternative source of Na and K in mixed rations.

7.5. Conclusion

Irrigation water quality significantly influenced the plant dry matter yield and some selected nutritional parameters. *M. arborea* showed reduced yields (2.8 t/ha/year) in 16 dS/ m water salinity but *Atriplex* species yield was higher for the same condition. At 800 mm/year irrigation rate plants overall DMD, DOMD and ME were significantly reduced at 16 dS/m irrigation salinity. Collectively, NDF values of plants were reduced when the salinity increased from 0.8 to 16 dS/m in irrigation water. *Medicago arborea* had significantly higher NDF (36.0 % of DM) followed by *A. lentiformis* (34.1% of DM) and *A. halimus* (31.53 % of DM). Higher irrigation salinities resulted in higher Na^+ concentrations in the forage but K^+ showed opposite trend. While forage Ca^{2+} concentrations actually decreased with increasing irrigation salinity in *M. arborea* but not in *Atriplex* species. The ash content was higher in *A. halimus* (25.1 % of DM) compared to *M. arborea* (7.5 % of DM). Based on the tested nutritional values these plants possess all the minerals that are required for ruminant diet. As *Atriplex* species had high tissue Na content it may not be suitable to use as a sole form of diet for the ruminant but during forage shortage season, *Atriplex s*pecies can be an alternative source of food for ruminant if given in a mixed ration. In addition, its ability to produce higher biomass in a saline environment gives the farmer an opportunity to produce valuable feed from degraded land where nothing else may grow.

Chapter 8: General discussion

This chapter highlights the results of the previous sections with a boarder outlook. Consideration will be also given to the issues not examined by this research that are necessary to investigate for the commercial use of saline agriculture.

The shortage of the fresh water for agricultural production is accelerating and this is limiting crop production in many parts of the world, particularly eastern Mediterranean countries and Australia (Ventura et al., 2014; Schacht and Marschner, 2015). Increased shortage of freshwater (EC_w<0.8 dS/m) has led to the use of low quality water (saline water) worldwide, especially in arid and semi-arid regions (Abou-Hadid, 2003; Stenhouse and Kijne, 2006; Bauder et al., 2008; Huang et al., 2011). Saline waters may include reclaimed waste water, saline drainage water and industrial saline water. Some of this saline water is produced as a by-product of the operation of gas and oil industries and thus can be a potential source for the irrigation. For example, in 2013, Coal Seam Gas (CSG) operations produced approximately 18,500 megalitres of wastewater with salinity levels typically up to 17 dS/m (IESC, 2014). However, this water cannot be directly applied to conventional crops without significant yield penalties. Its application also raises concerns about potential increase in soil salinity and sodicity. Preventing increased soil and land salinisation by use of large scale saline water resources requires ongoing research and development to protect to the greater environment.

This study of short-term glasshouse based responses and longer term (3 years) field investigations has shown the impact of different concentrations of saline water on soil properties and plant yield and quality. To investigate the impacts of different soil textures, field soils, salinity levels and irrigation rates glasshouse and field experiments were established to measure plant biomass production across a range of plant species. These experiments provided a good opportunity to understand how the different plants interact with saline water and evaluate the impact of saline irrigation on soil chemical properties.

The results of glasshouse experiments have shown that there are significant effects of soil texture and salinity levels on plant yield. There were no significant effects of irrigation rate on the plant yield suggesting both irrigation rates provided optimal

water. The salt tolerant glycophyte (*Medicago arborea)* yield was significantly lower at the high salinity level (beyond 8 dS/m) and their production under these conditions would be economically unsustainable. The *Atriplex species* performed better in the saline compared to non-saline conditions (Chapters 3, 5 and 7). The salinity of the irrigation water was a major factor determining yield potential and yield differences between plant species. Similarly, in field experiments *Medicago arborea* grew satisfactorily up to an irrigation salinity of 8 dS/m while *Atriplex* were unaffected at 16 dS/m. As such, *Medicago arborea* is recommended for growing under conditions where water salinity levels are less than 8 dS/m, while *Atriplex* species can be grown at water salinity levels of 16 dS/m without affecting biomass production.

A short-term glasshouse experiment demonstrated lower plant yield in a sandy loam compared as compared to a clay textured soil. This suggests that soil texture and thus inherent soil nutrient levels play a significant role in plant performance in saline situations (Chapter 3). The reduction in yield of *M. arborea* with increased levels of salt in the irrigation water indicated they were suffering from either or both osmotic stress and specific ionic toxicity in saline environment. This is also supported by increased leaf Na^+ and reduced stomatal conductance and leaf K^+ content of those plants grown in saline treatments. The reduction in stomatal conductance limits both net photosynthesis (Flowers et al., 2015) and photysynthates availability for plant, as a result plant growth and development became slow and yield declines (Flowers and Colmer, 2008). The higher yield of *A. lentiformis* in high salinity water indicates that these plants are equipped with highly efficient mechanisms to ensure efficient control of stomata (hence, sustained CO_2 assimilation) under saline conditions.

The halophytes *A. lentiformis* had a higher leaf Na^+ and K^+ concentration than the salt tolerant glycophyte *Medicago arborea* but still yielded well. The K^+ retention ability of plants has been linked to the salinity tolerance (Chen et al., 2008; Smethurst et al., 2008) because K^+ is necessary for the activation of over 50 enzymes (Marschner, 1995), including those involved in carbon fixation (Jin et al., 2011). Thus reduced cytosolic K^+ can negatively affect cell metabolism (Shabala and Cuin, 2008), protein synthesis (Weber et al., 1977) and operation of the tonoplast H^+-PPase (Belogurov and Lahti, 2002; Gaxiola et al., 2007). It is therefore important to maintain high cytosolic K^+ (~100-150

mM) concentrations in the cytosol for the maintenance of photosynthesis. This notion is supported by the findings in Chapters 3 and 4 where salinity tolerant plants (*Atriplex lentirormis*) maintained high leaf K^+ concentration. Similarly, the hallmark traits of salinity tolerance exhibited by halophytes is the accumulation of higher concentrations Na^+ in their shoots (Yamaguchi and Blumwald, 2005; Shabala, 2013) compared with less tolerant plants (glycophytes). Halophytes use Na^+ to act as an alternate osmoticum for osmotic adjustment in saline environment (Shabala and Mackay, 2011; Hariadi et al., 2011) and reduce the need of ATP for this purpose, thus allowing plant growth and development to be continued. However, this substitution has its limits and so as a plants tolerance is reached further increases in cytosolic Na^+ or Cl^- concentrations begin to affect the plant metabolism. This process is also accelerated by the low cytoplasmic K^+, Ca^{2+}, Mg^{2+} or other mineral deficiencies (Flowers et al., 2015). In addition to this decreased stomatal conductance and net photosynthesis reduces the energy required for ion transport and/or the synthesis of compatible solutes which thus reduce growth and eventual kill the plant.

An analysis of kinetics of ionic status in plants identified that the glycophytic plant *M. arborea* accumulated less Na^+ at the initial stage but could not limit its uptake once the plant was exposed in saline environment for a longer period of time (Chapter 4). At the same time, *A. lentifiormis* (a halophytic plant) had initially higher Na^+ levels but maintained them at constant levels throughout the growth period although concentration was as much as double of *M. arborea*. These patterns imply very efficient control over the xylem Na^+ loading in halophytes species (Shabala, 2013). The use of Na^+ as an osmoticum in halophytes also comes with a carbon cost advantage. Less salinity tolerant plants seem to be using organic solutes for osmotic adjustment. While the energy cost of pumping Na^+ back to the rhizosphere, which is mediated by the plasma membrane SOS1 Na^+/H^+ exchanger, and its vascular sequestration, which is mediated by NHX tonoplast Na^+/H^+ exchanger, is the same, in the former case plants to spend a significant part of the ATP pool for *de novo* synthesis of compatible solutes, for osmotic adjustment purposes. Thus, less ATP is available for other metabolic- and growth-related processes. This is further supported by the higher relative percentage of organic osmolytes in *M. arborea* (Chapter 4) compared to *A. lentiformis*.

High levels of salts in irrigation water significantly increased soil salinity but the effects varied based on changes in soil textures. Higher soil ECse values were observed in a high clay content soil. This indicates that clays finer pores held the salty water and restricted downward transport of salts (Brady and Weil, 2009) and not removed as easily as in the sandy loam soil. In glasshouse experiments the lower ECse values in soils irrigated at a higher rate indicated that application of sufficient amounts of water could be beneficial if the watertable is low and the soil has high permeability. However the lower ECse values (compared to EC_W) measured in irrigated field soils at the higher application rates indicates salts are being leached beneath 0.5 metre. Increased topsoil ECse, chloride and ESP values in 16 dS/m water salinity indicates that this level of salinity will dramatically impact the growth and yield of almost all crops. Unfortunately highly saline water caused the leaching of exchangeable base cations ($Ca^{2+,}$ Mg^{2+}, K^{+}) to below the active root zone (Chapter 6) making the upper profile sodic in the process and also induced nutritional imbalance in the soil. In addition, increased topsoil (<50 cm) sodicity (ESP) in 16 dS/m saline water indicates there will be a severe decline in soil porosity, infiltration and water holding capacity in rainfall events following cessation of saline water irrigation. This has significant implications for crop management and soil management. To avoid the yield penalties from this nutrient leaching nutrient base cations would need to be supplied in saline water irrigation soil.

The consistent results between glasshouse and field experiments observed for plant performance provides confidence that plant yield under saline irrigation will be repeatable in a predictable manner under the same soil and growth condition. *Medicago arborea* and *Atriplex* species have shown comparable nutritional values with conventional pasture e.g lucerne (Chapter 7). The salinity gradient has no or very small effect on the nutritional quality of the forage, except for elevated Na^{+} ion concentration in plants from the high salinity treatment. Overall, crude protein (CP), dry matter digestibility (DMD), dry organic matter digestibility (DOMD), and metabolisable energy (ME) values were affected at 16 dS/m salinity irrigated at 800 mm/year rate. However, there is little or no change observed in nutritional quality under saline irrigation when applied at 200 and 500 mm/year irrigation rate for the same salinity level. Hence, it

clearly shows that these plants have an important role to play in pastoral and agricultural enterprises in saline environments across the world.

Current government legislation (Environmental Protection Act 1994) prevents the use of marginal quality water for broad scale irrigation and does not take into account many factors such as, soil texture and structure, irrigation rate and plant types which can minimise the negative effect of saline water on soil physical and chemical properties. According to the Environmental Protection Act 1994, CSG water is considered as a waste product (as defined under section 13 of the EP Act). Depending upon the situation, other legislation such as the Water Supply Act (Safety and Reliability) Act 2008 (Water Supply Act), and the Waste Reduction and Recycling Act 2011 may also control the use of CSG water (DEHP, 2012). These regulations generally fail to fully account for the amount of Na^+ withdrawn from the soil by growing halophytic plants as highlighted by several authors (Pasternak et al., 1985; Zhao et al., 2005; Glenn et al 2009; reviewed by Panta et al., 2014). They also do not account for the amount of irrigation water used and soil texture. Indeed, at highest irrigation regimes (800 mm/year) the overall amount of salt measured in the present field study was 25% less than the amount of salt in the irrigation water indicating losses to deep leaching and plant uptake. Similarly, in glasshouse experiment ECse of sandy loam soil had three fold lower than that of EC of irrigation water. Although, the existing regulations (the Coal Seam Gas (CSG) Water Management Policy 2012) encourage the beneficial use of CSG water that meet the criteria set by the previous policy (Gasfields Commission Queensland, 2014) but it prevents conducting large scale field based research trials from untreated waters, even in the marginal lands, which are needed to fully investigate the productivity and environmental implications of reuse of saline irrigation waters. Without real in-field saline irrigation research it is not possible to achieve findings which are acceptable to the farming community.

There is a vital need of continued discovery-based research to address the ongoing issues for the saline agriculture. While high quality fertile lands ought not be used to grow halophytes, more marginal lands could be. Marginal saline, saline-sodic and sodic lands which already contain substantial amounts of Na^+ (sodicity) and NaCl (salinity) could grow halophytes using saline irrigation while remaining environmentally sustainable. As reviewed in chapter 2, some halophyte species may take up between one

and six tonnes of salt per hectare per year. This may be a beneficial for farmers to reclaim the saline or highly sodic land under halophyte cultivations. Thus, it is recommended that more flexible legislation is introduced to account for these factors.

The saline co-produced water may not only contain high concentration of Na^+ and Cl^- ions (that can be managed by halophytes) but also other elements e.g. boron, fluoride, silicate, and other chemicals that used for the fracking process (Xu and Drewes, 2006; Fakhru'l-Razi et al., 2009). Such Coal Seam Gas (CSG) waters may also contain substantial amounts of organic substances such as polycyclic aromatic hydrocarbons e.g., benzo[a]pyrene, dibenz[a,h]anthracene, fluorene, benz[a]anthracene (Tang et al., 2015), phenols(2-Chlorophenol, 2-Nitrophenol, 4-Chloro-3-methylphenol) and total hydrocarbons (nC10/nC14, nC15/nC28), radionuclides and heavy metals (Shaw, 2010; Stearman et al., 2014). This may have some significant impact on the plant growth and forage quality. Plants grown using such waters may potentially accumulate toxic molecules/elements in their tissue at the elevated levels. Then, if these plants were given to domestic animals, as an alternative source of fodder, they may cause some health related issues in animals and associated products. Thus assessments of animal health and thus feeding trials are crucial because higher molecular polycyclic aromatic hydrocarbons (PAHs) may cause cancer through metabolic activation in the cells (Melendez-colon et al., 1999; Luch and Baird, 2004; Xue and Warshawsky, 2005).

So far this study has shown the production potential of the *Atriplex species* and *Medicago arborea* and their possibility for use as alternative source of fodder (based on analysis of nutritional quality) and has opened up the opportunity for saline farming in sandier soils such as sands and loamy sands.

Leaf sap and tissue analysis of the studied plants revealed the status and rate of ion accumulation. However just how these other elements affect the plant performance and to what extent plant will uptake these elements needs to be further studied. The impacts on the soil microbiology and fauna also need investigating. Further considerations required include the economic aspects of halophytes cultivation. Are they equally sustainable in all soil types for long term farming?

Overall there is a dire need for research using the actual water coproduced by CSG operations (with different salinity) under field conditions using a wider range of soil

types. The research outcomes from experiments using only potting mixture or nutrient solutions do not represent the real growing environment for plants in the field or with real soil materials. Also the detrimental effects of salinity also depend on the duration of exposure and interacting effects of other stresses e.g. drought or waterlogging; so these aspects require further study.

Researching the merits of using saline agriculture in the modern world, one with increasing water shortages is critical to making informed decisions on their broader impacts and benefits. The most important point is the intended production region should have right soil types (sandier soils - preferable) and that the plant salinity tolerances match the salinity of irrigation water. It is hoped that the results of this study assist end users i.e. farmers to be more confident using saline waters of irrigation and for the government agencies to implement guidelines and regulation aiding saline agriculture adoption in the farming community.

Chapter 9: References

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